



# ANIMAL HEALTH BULLETIN

2010

**ROYAL GOVERNMENT OF BHUTAN**  
**MINISTRY OF AGRICULTURE AND FORESTS**  
Department of Livestock  
National Centre for Animal Health  
Serbithang, Thimphu

## TABLE OF CONTENTS

<b>CONTENT</b>	<b>PAGE</b>
FORWARD	i
ANIMAL HEALTH SERVICE IN BHUTAN	1
NEWS HIGHLIGHTS ON ANIMAL DISEASES 2010	2
1. Foot & Mouth Disease (FMD) outbreak	2
2. Detection of Reproductive & Respiratory Syndrome in Bhutan	3
3. Review of Gid Disease in Haa, Wangdue and Trashigang	5
4. Control of Animal Rabies in Bhutan	6
5. The Highly Pathogenic Avian Influenza (HPAI) outbreak February – March 2010	8
6. Prevalence of hypodermosis or warbles in yak	12
7. Study on Warbles in yaks in Merak and Sakteng	14
8. Prevalence of fasciolosis in Livestock population of Bumthang	16
9. Review of parasitic control schemes at NJBC, Samtse	16
10. Survey of Helminths in stray dog population	18
11. Wild Bird surveillance in Bhutan	19
12. Outbreak of Fatty Liver Haemorrhagic Syndrome (FLHS) at Kanglung	20
13. First outbreak of Peste des Petits ruminants (PPR) in Chukha Bhutan	22

14. Johnes Disease in Bhutan	24
15. Field Application of FMDV-NS ELISA	26
16. National Dog Population Management and Rabies Control Programme	29
<b>NEWS AND UPDATES</b>	31
17.1. Bird flu risk assessment study at West Bengal	31
17.2. Awareness on rabies for Medical and livestock officials	32
17.3. Teaching on HSI protocol in dog sterilization for CNR block week	32
17.4. Dog sterilization program at Serbithang and Babesa	33
17.5. Creation of Drug Vaccine & Equipment Unit (DVEU)	33
17.6. Rapid diagnosis of Rabies using RICT kits	34
17.7. Crisis Management Centre	35
17.8. Veterinary Laboratory Technology Diploma Course	36



## **FORWARD**

National Centre for Animal Health (NCAH) would like to produce the Animal Health Bulletin for the year 2010. This is the first edition which gives an overview of animal diseases based on the investigations and surveillance in the hope that it would provide the basic information on animal diseases in the country to all the readers.

The bulletin also highlights the short communications on important diseases and development.

On behalf of the NCAH staffs and management, I would like to thank all the officials and staffs for their valuable contribution and support in producing this bulletin. I would also like to thank all the authors within the organization for their contributions in the bulletin.

Dr. Karma Rinzin  
**Programme Director**



## **ANIMAL HEALTH SERVICES IN BHUTAN**

Animal health services are provided free of cost to the people by the Royal Government of Bhutan. Due to the inherent element of high cost animal health service, every effort has been made by the Royal Government to curtail the expenditure. Accordingly, a shift in the modality of health service delivery has been introduced in the eighth plan and to mention a few are the cost recovery for curative veterinary care, establishment of revolving fund for Drug Vaccine and Equipment (DVEU) and selective approach to preventive animal health care such as immunization and de-worming programmes. Most of the preventive health care is now based on epidemiological findings. The disease surveillance and early warning of any epizootics originating from the international border (southern side) are being investigated through the Regional and Satellite laboratories. Any livestock imported has to be quarantined at least for 14 days under the Livestock Legislation. This prevents entry of diseases from other countries.

Concomitant with the gradual increase in the number of improved breeds of livestock, the necessity to improve upon the quality of health care service is indispensable in the future given the proven fact that improved breeds are relatively more susceptible to disease problems endemic in the country. Disease susceptibility of these breeds is further aggravated by poor management, nutrition and sanitation at the village level. The emergence of multi-factorial diseases (mixed infections) has been found to be very high which reduce the productivity of the improved breeds of farm animals managed under poor condition. The promotion of indigenous knowledge on veterinary health care and re-enforcement on farmer's awareness on the importance of animal health care in increasing productivity is still in great need. Therefore, proper health care and management through efficient veterinary health care support remain a crucial component.

# NEWS HIGHLIGHTS ON ANIMAL DISEASES 2010

## 1. Foot and Mouth Disease (FMD) outbreak

DR. PASANG TSHERING, AHS, NCAH, SERBITHANG

FMD is endemic in Bhutan and mainly prevalent in the southern and central Districts of the country. There are around 12 outbreaks of FMD reported every year in the country. During the period of January to June 2010 there were 6 outbreaks of the disease. The first outbreak in the country was detected in Keptong village, Bartsam Geog, Trashigang Dzongkhag on 29<sup>th</sup> January. This was followed by an outbreak in Dairyland, Bhur Geog, Sarpang Dzongkhag in February. In March the disease was reported from Marangdut and Sonamthang locality under Panbang Dungkhag, Zhemgang Dzongkhag. Both cattle and pigs were affected in the Panbang outbreak and in other outbreaks only bovines were affected. In May outbreaks were reported from Samtse and Sarpang.

In Sibsoo Geog, Samtse Dzongkhag three buffaloes were also affected besides cattle. The source of the virus in the outbreak of Sibsoo could have originated from across the border as the disease was prevalent in the bordering Hiley village in India. The outbreak in Keptong village, Bartsham Geog, Trashigang was initially seen in two mithun crossbreeds and three Jersey crossbreeds.

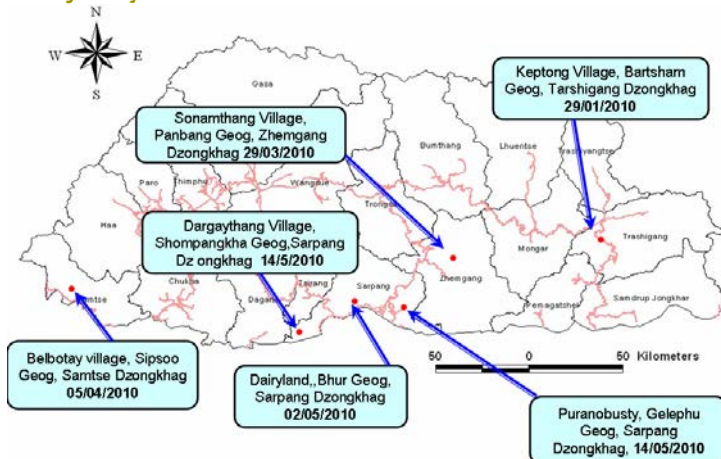


Fig 1: FMD outbreaks during January to December 2010 in Bhutan



The source of the outbreak in Panbang is implicated to be brought through meat (kanchungs or feet) of cattle brought from Phuentsholing or Gelephu.

---

## 2. Detection of Porcine Reproductive and Respiratory Syndrome virus (PRRSv) in Bhutan

DR. VIJAY RAIKA, PAHO, NCAH, SERBITHANG

Porcine reproductive and respiratory syndrome (PRRS) is a viral disease of pigs that emerged in the end of 1980s. The disease is endemic in most of pig producing countries but occasionally it is observed in epidemic form with high mortality. Recently highly acute PRRS outbreaks were reported from China and Vietnam (1). These outbreaks increased global interest about this disease, including Europe. The highly virulent strains presently circulating in China and Vietnam pose serious threat for the neighboring countries. Here we describe the first case of PRRS in Bhutan caused by the highly pathogenic variant of PRRSV.

On August 4, 2008, at National Pig Breeding Centre (NPBC), Wangchutaba, clinical symptoms resembling PRRS were observed in several sows. In August, 15 of 41 pregnant sows (36.5%) aborted on 52-109 day of gestation and two of them died. Three boars and two gilts also died. From August to October, 15 more sows (36.5%) gave birth to litters consisting of stillborn and weak piglets (Figure 1).



Post-mortem examination of the dead pigs showed congestion in lungs with interstitial pneumonia and hemorrhages in all organs. Twenty eight of 49 live piglets born in this period died before weaning. Serum samples from pigs from the affected herd were found to contain PRRSV specific antibodies. To characterize

Figure 2: Aborted and still-birth foetuses

PRRSV from Bhutan, sera from 30 pigs and organs from four fetuses and two dead pigs were submitted to the OIE Reference Laboratory for PRRS at the National Veterinary Research Institute, Pulawy, Poland.

Antibodies specific for PRRSV genotype 2 were found in sera from 14 pigs. By PCR PRRSV genotype 2 was found in two of these sera and in one sample from sero-negative sow. In three of six organ samples PRRSVs RNA was also detected. Results of PCR to detect specific 90 nt deletion in nsp2 coding fragment, considered the marker of pathogenic PRRSV strains from China and Vietnam confirmed presence of such deletion in the sequences from Bhutan. Sequence analysis of the amplicons showed that they were highly identical (99.7-100% at ORF5; 99.2-100% at ORF7). Their identity to the pathogenic strains isolated earlier in China and Vietnam (1) was also very high (98.8-99.7% at ORF5; 98.7-99.7% at ORF7). In the phylogenetic trees the sequences from Bhutan clustered together with those from China and Vietnam supports their common origin (Figure 2). The identity of the sequences from Bhutan to the prototype PRRSV genotype 2 strains VR2332 was much lower ranging 88.6-88.9% at ORF5 and 93.3-94.1% at ORF7. There was report on abortion and mortality from Gelephu Nucleus Pig farm also in April 2010. The sera from the farm were negative for PRRSV antibodies. However, PRRSV antigen was detected in one serum sample. The presented data indicated that PRRSV genotype 2 was detected in the two pig breeding farms (Wangchutaba and Gelephu) and suggested that the outbreak was caused by similar PRRSV strain detected in China and Vietnam.

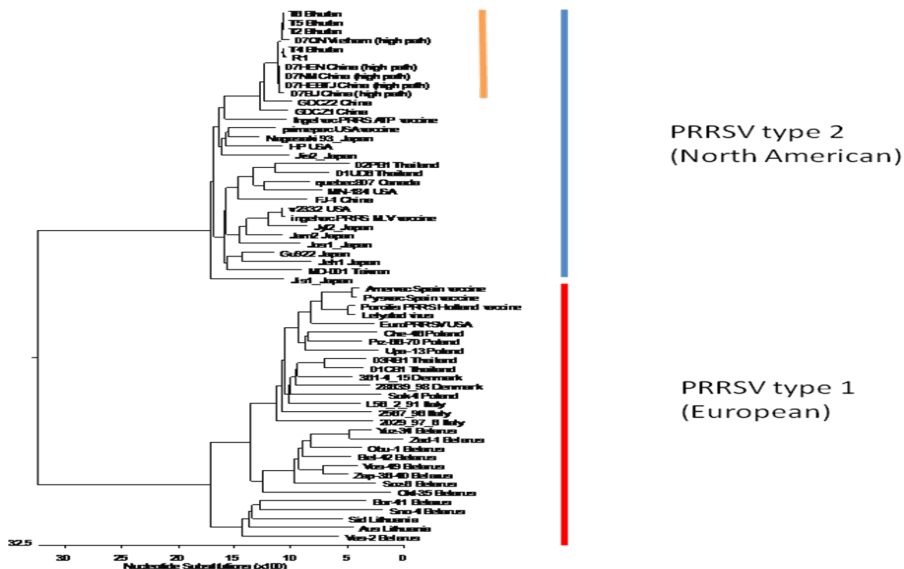


Figure 3: Phylogenetic analysis of PRRSV. The green vertical line indicates the clustering of PRRSV detected from pigs of Bhutan.

### 3. Review of outbreak of gid disease in Haa, Wangdue and Trashigang Dzongkhag in 2010.

Yak farming is an eco-friendly, economically important traditional technology in high altitude hilly ecosystem (Ramesha et al 2009). It is part and parcel of social and cultural fabric of tribal yak herdsman living in difficult hilly terrains of the Himalayas in particularly to Bhutan. But yak development is continuously hampered with constant outbreaks of gid disease or coenurosis in 1 to 3 years age groups of yaks (Wangdi 2008) mainly due to inaccessible to animal health support facilities and insufficient budget for the control programs. Thus in the present studies an attempt was made to follow up the gid disease situations in Haa, Wangdue and Trashigang Dzongkhags with the following results.

Recent study revealed that in three yak Gewogs of Haa, only two Gewogs

vide Katsho (69-11.53%) and Bjee (12-2.01%) was affected by gid disease. 81 yaks in 1 to 3 years age groups had died of gid disease resulting to an overall gid prevalence of 13.55% in Haa for 2009. While study in Wangdue showed an overall gid prevalence of 1.42%. This was resulted due to death of 2 young female yaks in a population of 132 yaks among 1 to 3 years of age (10 yak herders) in Sephu Gewog which was formally purchased from Haa. But the detail investigation studies in yaks and in golings showed that gid disease outbreaks had not occurred in Merak-Sakteng for the past many years. Thus gid disease is obviously reemerging in Wangdue Dzongkhag and purchase of yaks from gid yak Dzongkhag should be discouraged. In future gid disease status should be thoroughly studied in yak rearing Dzongkhags so that gid disease control program could be reviewed.



#### **4. Control of Animal Rabies in Bhutan**

DR. KARMA RINZIN, PROGRAMME DIRECTOR, NATIONAL CENTRE FOR ANIMAL HEALTH,  
SERBITHANG

Rabies represents a significant cost to the Bhutanese community due to the cost of control measures, loss of livestock, and human life. The cost of post exposure rabies treatment of humans that have been bitten by dogs has been at US\$ 0.070 million annually. Dogs are the main reservoir hosts for rabies in Bhutan. The government of Bhutan has assigned high priority to rabies control and is in the process of implementing a major control strategy designed to reduce the incidence of disease.

Various control measures were tried to effectively control rabies in Bhutan. Nationwide elimination of dogs as a means to control rabies was introduced in 1980s. Following this ad-hoc vaccination and sterilization of dogs was initiated to control rabies in Bhutan. The revamped rabies control programme was started from 2004 onwards. As a result of initiating various control strategies now the majority of the outbreaks were reported in districts adjacent to the southern border of the country only. The spatial distribution of rabies outbreaks in interior districts were consistent with the pattern of seasonal migratory routes taken by domestic animal species

(mainly cattle).

Rabies control documents was reviewed and amended from time to time. Some of the recent modifications based on World Health Organization (WHO) & World Society for Protection of Animals (WSPA) expert recommendations are a) control of habitats; ii) legislative measures and iii) animal birth control measures. Department of Livestock under Ministry of Agriculture is carrying out advocacy campaign on the control of dog habitats to the general public through mass media, public meetings and sensitization meetings of the stakeholders. Besides, Department is also closely working with Municipal Corporation on the management of solid wastes in major cities. Rabies Control Regulations was prepared with the support of international expert fielded through the WHO supported Project on Control of Rabies in Bhutan in year 2007. The regulations outlines the roles of a responsible dog ownership, role of municipal corporation and general public in control of dog habitats and management of stray dogs. Since the animal birth control (ABC) is a proven technique to control the dog population and rabies, Department of Livestock under Ministry of Agriculture in collaboration with Humane Society International (HSI) is now carrying out a nationwide Capture, Neuter, Vaccinate and Release (CNVR) Programme. The techniques adopted in the current CNVR programme is technically and scientifically accepted by international scientific communities and humane societies. The success of the programme is monitored and evaluated using a wild life technique called “Capture-Mark-Recapture” Technique. This technique allow us to evaluate the CNVR programme as well as to estimate the approximate free roaming dog population in the areas. This joint programme will be seriously carried out for a period of three years and will be continued if felt necessary.

In year 2010 total 15 rabies outbreaks were reported from different parts of the country (Refer Figure . Of the 15 rabies outbreaks 10 outbreaks are reported from Chhukha Dzongkhag. Most of the reported outbreaks are along the southern international borders. The outbreak in the interior parts of the country such as Logchina Geog in Chukha Dzongkhag was in a horse which had an exposure at Phuentsholing while using it for

transportation of goods from Phuentsholing to Logchina. All the outbreaks are efficiently managed and controlled by the respective dzongkhag Livestock Sector and Regional Livestock Development Centres.

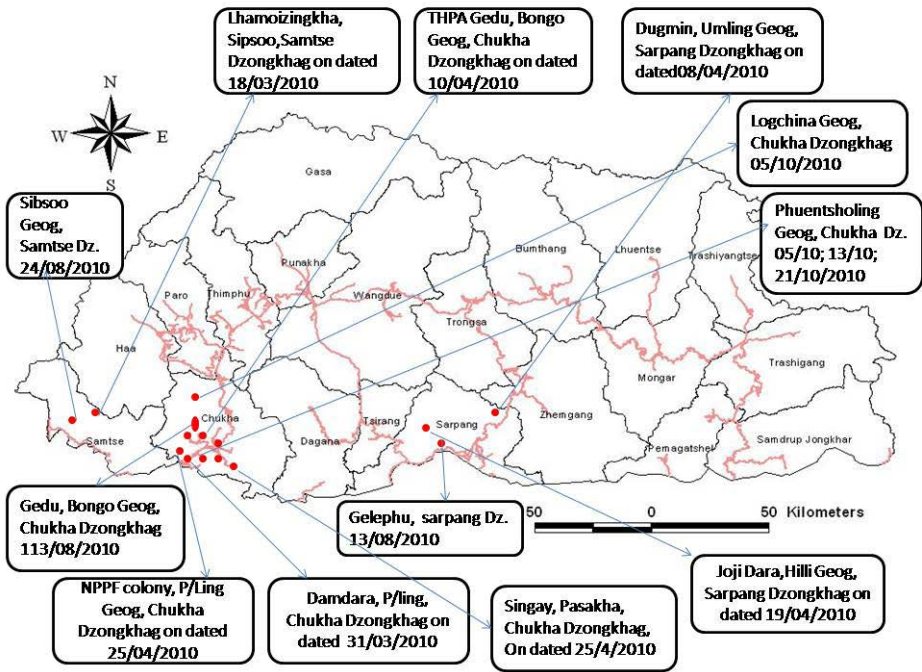


Figure 4: Rabies outbreaks during January to December 2010.

## 5. The Highly Pathogenic Avian Influenza (HPAI) outbreak February – March 2010

DR. KARMA RINZIN, PROGRAMME DIRECTOR, NATIONAL CENTRE FOR ANIMAL HEALTH, SERBITHANG

### *The HPAI outbreak, February – March 2010*

All of the poultry affected in the outbreak were ‘scavenger’ chickens: all chickens culled were in sector 4 management systems. The index case was reported on 17-Feb-10 at Rinchending, Phuentsoling Geog (block or sub-district) adjacent to the main road in a Royal Bhutan Police colony

lived in by officers who man the adjacent road check point with Bhutan Agriculture and Food Regulatory Authority (BAFRA) officers. The map that follows shows the Geogs in Chukha Dzongkhag. The outbreak was in Phuntsoling and Samphelling Geogs.



*Figure 5 : Map of Chukha Dzongkhag showing Geogs (red dots indicates outbreak location in the affected Geogs).*

Seven birds died out of eight that were visibly affected at Rinchening. Clinical signs were consistent with HPAI. Samples for laboratory confirmation were taken from birds that had been opened for post-mortem examination. There was a positive rapid test result on-site. The signs and mortality were indicative of HPAI and so the authorities culled all 28 birds in this colony on 20-Feb-2010 before laboratory confirmation was received on 22-Feb-10 evening.

Tracing suggested that at least one bird had been purchased from

residents of an industrial area housing colony at Pasakha, near to the international border, some five kilometres from Rinchending. On 19-Feb-10 three chickens died at Pasakha Bazaar. This mortality was picked up by a Veterinary Vigilance Team (VVT). These deaths were subsequently confirmed as HPAI.

On 25-Feb-10 three birds died in Toorsa valley area, west of the Rinchending/ Pasakha areas. Clinical signs were suspicious and control measures were carried out (see below), although laboratory tests identified Newcastle disease virus (NDV) as the cause of this mortality.

On 02-Mar-10, 15 chickens died within two days in Damdara, close to the index case at Rinchending. The birds were rapid test negative, but full control measures were again carried out.

On 11-Mar-10 two chickens died at Ramitey, also close to the index case. These were rapid test positive, but the carcasses were disposed of in a pit and samples were not collected for laboratory confirmation.

On 14-Mar-10, two chickens died at Burkhey, a few kilometres east of the index case. The mortality was reported by the 'Village Focal Person', followed up and samples subsequently confirmed by RT-PCR at the NCAH laboratory in Serbithang, Thimphu.

Thus, there were three confirmed 'epicentres' in this outbreak: Rinchending, Pasakha and Burkhey. For the other three locations, Toorsa was shown to be NDV, Damdara was a suspected case negative on both rapid test and PCR at NCAH, and Ramitey was positive with rapid test but samples were not retained for laboratory confirmation. Damdara and Ramitey were closely associated with Rinchending.

Pasakha Bazaar and the Rinchending Royal Bhutan Police colony have the workers' housing near to a main road. All other locations comprise scattered, rural households in hilly areas that are only accessible by small footpaths.



In response to the outbreaks, more than 5000 chickens were culled and around 800 eggs were destroyed. Coops and wooden housing was burned and the sites were disinfected. After 14-Mar-10, no further mortality was reported in the area and no poultry mortality reported in other Dzongkhags was confirmed as HPAI.

The map below shows the approximate locations of the confirmed and suspected HPAI cases.

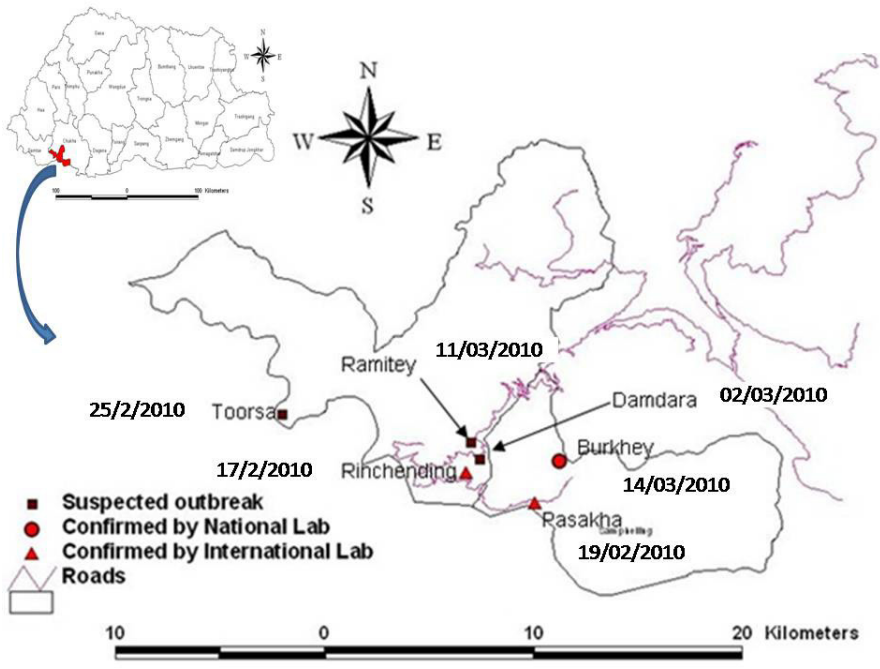


Figure 6: Locations of the confirmed and suspected outbreaks in two Geogs (Phuentsoling and Samphelling) of Chukha Dzongkhag



(a)



(b)



(c)



(d)

*Figure 7 : Typical clinical signs and lesions (a) swollen head and eye lid (b) cyanosis comb and sudden death (c) haemorrhages of the shank region (d) subcutaneous haemorrhages*



## **6. Prevalence of hypodermosis or warbles in yak**

DR. PHUNTSHO WANGDI, AHS, NCAH, SERBITHANG

Hypodermosis or warbles are a somatic myiasis caused by larval stages of *Hypoderma* species of flies (Diptera, Oestridae). It is recognized as one of the major arthropod parasitic infection inducing huge economic losses to cattle industries as well as in yak rearing areas worldwide since three centuries back (Zumpt. 1965). Recent reports indicated that in China, hypodermosis is also considered as one of the most important parasitic diseases affecting ruminants (yaks and cattle) and moreover reports of *Hypoderma* species affecting farmers.

Results from preliminary studies also showed that hypodermosis is well

established in many rearing areas of Bhutan and it was reported by many aged old yak herders that warbles was present with yak herds since their childhood since early 1950's. Preliminary studies from revealed the hypodermosis prevalence at 15.50 %, 71.70%, 95.00%, 45.36%, 39.25% and 54.44% for Bumthang, Gasa, Haa, Paro, Wangdue and Trashigang respectively. It is also well understood that yak rearing is the only means of livelihood in majority of these areas. For this reason a Warbles control program was developed and implemented since late 2009.

*Table 1. Warbles or Hypodermosis control program activities in yak rearing Dzongkhags: Highlights of achievements for fiscal year July 2009 to June 2010:*

Dzongkhag (1)	Gewogs (2)	Warbles (3)	Control activities		
			Prophylactic (Ivermectin) (4)	Yak Herders training (5)	Awareness campaign (6)
Haa	Katsho	97.94%	-	35 herders	-
	Bjee	86.83%	-	"	-
	Eusu	100%	-	"	-
Paro	Soi yaksa	50.94%	-	-	-
	Nubri	39.79%	-	-	-
Thimphu	Soi	-	-	22 herders	-
	Lingshi	-	-	30 herders	-
	Naro	-	-	25 herders	-
	Dagala	-	-	20 herders	-
Gasa	Laya	51.90%	4709	-	51 herders
	Lunana	91.50%	2731	-	"
Wangdue	Sephu	34.30%	-	147 herders	-
	Phobji	45.10%	-	"	-
	Gangtey	35.30%	-	"	-
	Kazhi	41.40%	-	"	-
Trongsa	Nubi	-	-	-	-
Bumthang	Chokhor	15.50%	-	-	-
	Chumey	-	-	-	-
	Tang	-	-	-	-
	Ura	-	-	-	-
Lhuntse	Khoma	-	-	-	-

T.yangtse	Bumdaling	-	-	-	-
Trashigang	Merak Sakteng	30.48% 23.95%	3063 1480	150 herders “	- -
<b>Total</b>	<b>24 No's</b>		<b>14714</b>	<b>429 herders</b>	<b>51 herders</b>

## 7. Study on Warbles in yaks in Merak and Sakteng under Trashigang Dzongkhag

DR. PHUNTSHO WANGDI, AHS, NCAH, SERBITHANG

Recent studies in hypodermosis problem at yak rearing locations of Merak and Sekteng revealed an astonishingly higher warbles prevalence of 54.43%, which was caused due to infection of 11,206 yaks and golings. Studies also showed that 5693 yaks and 5513 golings were infected resulting to 27.65% and 26.78% prevalence rate respectively. The results thus indicated that both yaks and golings of all ages are susceptible to Hypoderma larvae infection and are equally distributed in both the breeds.

The overall warbles prevalence were estimated at 54.88% and 53.86% resulted from infection of 6247 and 4959 yaks and goling in Merak and Sekteng gewogs respectively. Studies also revealed warbles prevalences as 30.48% and 30.20% due to infection of 3188 and 3059 golings in Merak. While in Sakteng 2505 yaks and 2454 golings were infected giving rise to 23.95% and 24.23% warbles prevalence. Thus the research findings showed that yaks and golings are equally infected in both the gewogs. Studies also revealed that Hypoderma larvae infection had already spreader to 6 cattle herds of Thrakthri village of Sakteng gewogs resulting to 24.12% warbles prevalence. Based on the findings preventive measures were recommended for immediate imprecations including chemoprophylaxes and chemotherapeutic medications along with surgical treatment of warbles on warbled yak rearing gewogs of Trashigang.

*Table 2: Total yak and goring population, Total yaks (both adult and young) and golings (both adults and young) infected with warbles, total warbles prevalence in both adults and young yaks and golings in Merak and Seking Gewogs:*

Merak	Yaks		Goling		Total	
	Total	Infected	Total	Infected	Total	Infected
Tot A/Y pop	58.49	3188	5532	3059	11381	6247
Warble pre	-	30.48%	-	30.20%	-	54.88%
Tot A pop	3101	1611	2644	1260	5745	2871
Warble Pop	-	15.40%	-	12.44%	-	25.23%
Tot Y	2748	1572	2888	1799	5635	3376
Warble Pre	-	15.03%	-	17.76%	-	29.67%
<b>Seking</b>	-	-	-	-	-	-
Total A/Y Pop	4609	2505	4597	2454	9206	4959
Warble pop	-	23.98%	-	24.23%	-	58.86%
Total A Pop	2188	1117	2303	1127	4491	2244
Warble Pre	-	10.68%	-	11.13%	-	24.37%
Total Y Pop	2421	1388	2294	1327	4715	2715
Warble pre	-	13.27%	-	13.10%	-	29.49%
<b>Merak Sakteng</b>	-	-	-	-	-	-
G.Total A/Y Pop	10458	5693	10.129	5513	20587	11206
G.warble pre	-	27.65%	-	26.78%	-	54.43%
G.Tot A Pop	5289	2728	4947	2387	10236	5115
G.warble Pop	-	13.25%	-	11.60%	-	24.85%
G.Y Pop	5169	2965	5192	3126	10350	6091
G.warbles Pre	-	14.40%	-	15.18%	-	29.58%

**NB. A=Adults, G=grand, Pop=population, pre= prevalence, Tot= Total and Y=young**

## **8. Prevalence of fascioliasis in Livestock populating of Bumthang**

DR. PHUNTSHO WANGDI, AHS, NCAH, SERBITHANG

Fascioliasis, a fatal and economically most important Trematode parasitic infection, is caused by *Fasciola hepatica* or *F. gigantica* in livestock animals all over the world (Lloyd et.al 2001). In 2008 Food and Agricultural Organisation (FAO), had reported that more than 300 million bovines and 250 million sheep are exposed to the disease globally causing economic losses amounting to more than US\$3.2 billion per year. In addition fascioliasis is now recognized as an emerging human disease with 180 million people at risk (WHO 2009).

The annual laboratory reports from NCAH and RLDCs also indicate that fascioliasis is very much prevalent in different livestock species in the country (Parasitology Lab reports 2003-2008). Based on this, a preliminary survey of fascioliasis outbreaks was initiated in cattle population of Bumthang.

Preliminary study of 545 cattle herds of Bumthang showed that 391 herds (71.74%) were infected with *Fasciola*, indicating that majority of the cattle populating are exposed to fascioliasis. 86, 201 and 104 herds of Chummey, Chokhor and Tang are infected while no infection is revealed in 102 herds of Ura. Study also revealed that in cattle population of 5261 sampled, 2050 animals were positive to *Fasciola* species, estimating to an overall fascioliasis prevalence of 38.96 %, 16.51 % and 0.0 % respectively for Chummey, Chhokhor, Tang and Ura gewogs. Comparatively Chokhor showed higher parasitic prevalence of 16.51% while no *fasciola* species infection was recorded in cattle herds of Ura. Based on these findings it important to develop and implement a specific fascioliasis control programme for Bumthang Dzongkhag in future.



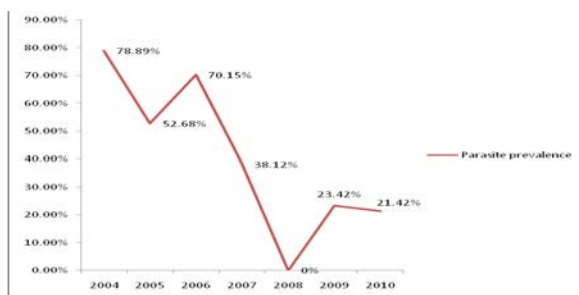
## **9. Review of parasitic control schemes at National Jersey Breeding Centre, Samtse**

DR. PHUNTSHO WANGDI, AHS, NCAH, SERBITHANG

Review studies in lab Based Deworming Programme (Chemotherapeutic strategies) and General Prophylactic Deworming Schemes

(Chemoprophylactic Strategies) revealed that overall parasitic prevalence had decreased from 38.12% in 2007 and 23.42% in 2009 to 21.42% by March 2010. The current prevalence of 21.42% is reported to have had caused by 16 cases of B.coli, 12 cases of Coccidia species and 15 cases of Strongyled Nematode species in 126 jersey animal sampled . Test results also showed that parasitic prevalence were 7.94%, 7.14% 12.69%, 0.79% and 4.79% respectively in Bulls, Heifers, Milch cows, Dry cows and Calves. Comparatively high prevalence of 12.69% was recorded in Milch cows than other groups in particular in dry cows with lowest prevalence of 0.79%. Higher prevalence in Milch cows (12.69%) was revealed to be due to nonpathogenic protozoan parasitic infection, by B.coli (6 cases out of total 9 cases).

Anthelmintic resistant tests (fecal egg reduction test) for the common dewormers in the farm vide Albendazole and Oxyclonazide was also initiated. Test results showed that anthelmintics efficacy of 80% (with B.coli) and 100% (without B.coli) respectively. Thus, indicating that Albendazole and Oxyclonazide are still effective against Nematodes and Trematode parasitic infections in farm animals. Despite the fact that overall parasitic prevalence had decreased from 38.12 in 2007 to 21.42% by March 2010, it would be necessary for the modification and further improvement of the existing deworming schemes in NJBC, Samtse for constant monitoring and evaluation of the parasite control schemes as well as to keep the parasites prevalence 2% and below. But it is doubtful whether Albendazole and Oxyclonazide have any effect on B.coli infection. For this more studies need to be performed in farm animals.



*Figure 8:Prevalence of parasitic infection (internal parasites in farm animals of NJBC, samtse from January 2004 to March 2010*

## 10. Survey of Helminths in stray dog population

DR. N. K. THAPA, PAHO, NCAH, SERBITHANG

Survey was conducted in stray dogs to know the prevalence of different types of helminth parasites and also to have a base line data for other zoonotic heminthiasis like Toxocariosis, Ancylostomiasis and Echinococcosis. The fecal samples were collected from the dog pounds and also during sterilization campaign conducted by Humane Society International (HSI) in Thimphu.

364 fecal samples were collected from 8 dog pounds from different parts of the country - Ngalephu, Thimphu (36), Gelegphu, Sarpang (60), Tshimasham (33), Toribari, P/ling (36), Dungbe, Zhemgang (32), Memlakha, Thimphu (50), Tshento Paro (37) and Stray dogs in Thimphu (80) and were examined for different types of helminthes.

Laboratory findings showed the prevalence rate of 43.7 % hook worms, 18 % Toxocariosis, 12.5 % Troglotrema, 9.5 % Coccidia, 2.9 % Trichuris and < 1 % others tape worm and Diphylobothrium. (Fig.1)

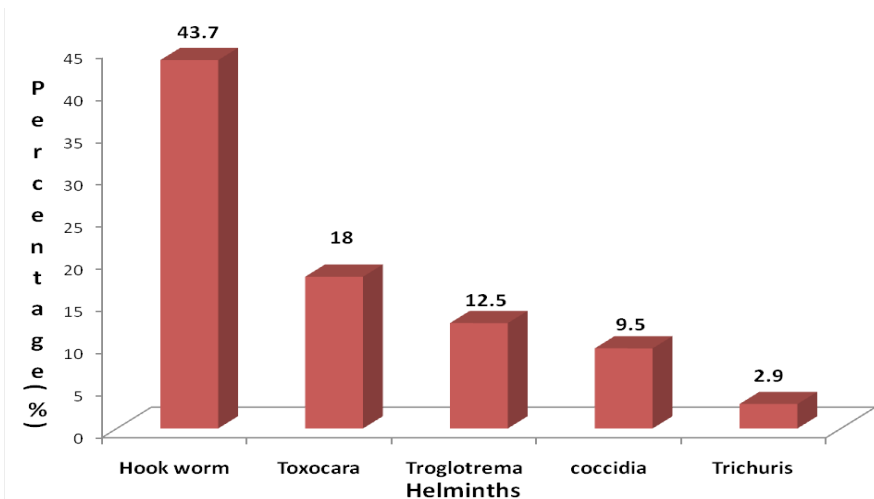


Figure 9:Prevalence of different helminthes in stray dogs

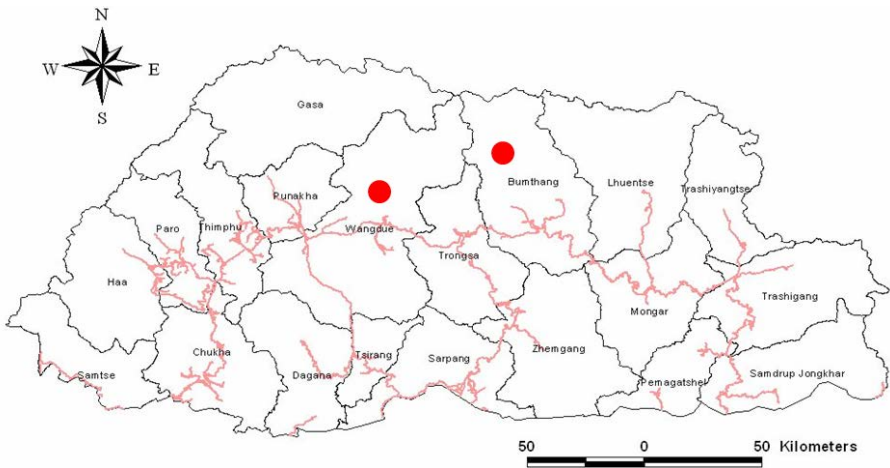
Samples of Dungbe, Zemgang revealed very high worm load of Ancylostomum with 500-2000 EPG. It was also detected in other areas except Ngalephu. Samples of Memelakha revealed very worm load of Toxocara spp. with 200-800 EPG and also hook worm of 200-900 EPG.



## 11. Wild Bird surveillance in Bhutan

DR. PASSANG TSHERING, AHS, NCAH, SERBITHANG

Bhutan is host to more than 770 species of birds and more than 165 species of mammals, with many species being endemic to Bhutan. Around 24 of these bird species are endangered and under conservation program in the country. About 40 species of birds migrate to Bhutan especially during winter. Bhutan falls on the Central Asia Flyways of the migration of birds. Most prominent migratory wild birds are the Black Necked Cranes (BNC) and some duck species which come to Bhutan during winter (October) and fly back as the summer approaches (February).



*Figure 10: Sampling sites for wild birds for investigating against Avian influenza during January 2010*

A Risk assessment for HPAI carried out in 2004 indicated migratory water birds to be a risk next to trade in poultry/poultry products. Therefore, clinical and laboratory surveillances were initiated since 2005. We mainly focus on clinical surveillance as it is easier and more practical. Laboratory surveillances is also planned to be conducted atleast once a year during the stay of the birds in the country. Mostly targeted sampling from high risk areas is carried out and also during sampling from domestic birds. The Department of Livestock works closely with Nature Conservation Division of Department of Forest and Park Services and Royal Society for

Preservation of Nature (an NGO) in surveillance works in wild birds.

During January 2010 clinical and laboratory surveillance was conducted mainly on the Black Necked Cranes and also some other water birds in Bumthang and Phobjikha. A consultant from AFRIMS



*Figure 11: Black-necked crane in Phobjikha*

(Armed Forces Research Institute of Medical Sciences based in Bangkok, Thailand supported the surveillance and accompanied during surveillance programme. Fresh faecal droppings were collected from four black necked cranes and two Ruddy shell ducks from Bumthang and 10 BNC from Phobjikha. All the samples along with 67 samples from poultry and domestic ducks were sent to St. Jude Children's Research Hospital, USA through AFRIMS. The samples were tested with real-time RT-PCR. All samples tested negative for Influenza A.



## **12. An outbreak of Fatty Liver Haemorrhagic Syndrome (FLHS) at Kanglung: a short communication.**

DR. S. B. CHAMLING RAI, AHS, RLDC, KANGLUNG

An outbreak of FLHS in a private poultry layer farm at Namla, Kanglung was investigated. From the least available records and enquiry with senior veterinarians, it is presumed that this is the first recorded FLHS with very high mortality encountered in Bhutan. The typical pathognomonic lesions observed were extremely enlarged liver (almost occupying whole of the abdominal cavity) and large amount of fat deposit in abdominal cavity (refer photographs). High energy diet, as suspected to be the cause, was proven indirectly, through a correction of the syndrome by a change in the commercial feed fed (layer ration sourced from different company).

FLHS being a new entity as an emerging miscellaneous management

related disease in commercial farming, a short academic communication is being made for the benefit of all concerned with poultry husbandry.

Fatty liver hemorrhagic syndrome (also referred to as fatty liver syndrome), is a disease in chickens and other birds, affecting only hens. Birds with this disease have large amounts of fat deposited in their liver and abdomen. This often results in an enlarged soft liver that is easily damaged and prone to bleeding. In some cases the disease is fatal, usually as a result of blood loss from an internal hemorrhage in the liver. The liver contains many blood vessels that rupture easily during straining to lay egg, resulting in massive bleeding and death.

Excessive dietary energy intake is believed to be the cause of fatty liver hemorrhagic syndrome. When laying hens are fed diets containing high levels of dietary energy the hens tend to deposit excess energy as fat deposits in their bodies, especially the liver. The problem is more common when feeds containing high levels of corn or other high energy ingredients are fed.

The research data suggest that defects in the hen's blood clotting system may be a factor triggering FLHS. The damaged blood vessel releases what is called a tissue factor (TF), which initiates the series of biochemical reactions leading to the formation of a blood clot. The data indicated that there was a significant difference in the production of thrombin, blood clotting protein, in normal versus FLHS birds.

Generally the disease has few or no symptoms prior to the bird's death. The condition is most often seen in birds that appear to be healthy and in a state of high egg production. As a result, death can occur quite unexpectedly. Non-laying hens will not eat as much of the high-energy feed and therefore are not affected as much as high producing hens.

The problem can be prevented by feeding complete layer diets that contain the proper amounts of all nutrients. In our context, high energy ingredients are corn and oil cakes. Thus, corn and oil cakes, excellent ingredients for poultry diets, should not exceed the standard proportion

in feed preparation.



*Enlarged Liver*



*Soft friable liver*



*Excessive at deposit in abdomen*

*Figure 12: Lessons of fatty liver haemorrhagic syndrome*

### **13. First outbreak of Peste des Petits ruminants (PPR) in Chukha Bhutan**

DR. BASANT SHARMA, RVO, RLDC, TSMASHAM

Peste des petits ruminants (PPR) is an acute or sub-acute and highly contagious viral disease of goats and sheep characterized by fever, oculo-nasal discharges, necrotic stomatitis, gastroenteritis, and pneumonia with foul offensive breath. It is also known as Pseudo Rinderpest or pest of small ruminants. It was first reported in the Ivory Coast in 1942 and subsequently in Senegal, Ghana, Togo, Benin, and Nigeria. Sheep are less susceptible than goats; cattle are only sub-clinically infected. Infected animals present clinical signs similar to those of Rinderpest in cattle, from which it must be differentiated. Because of the respiratory signs, PPR can be confused with contagious caprine pleuropneumonia (CCPP) or pasteurellosis.

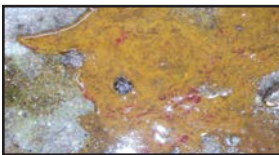
Peste des petits ruminants virus (PPRV) is a member of the genus Morbillivirus in the family Paramyxoviridae. Four genetic lineages (lineages 1-4) have been identified. PPRV is closely related to rinderpest virus. Among domesticated animals, peste des petits ruminants is primarily a disease of goats and sheep and wild ungulates. Cattle are usually infected asymptotically, and are not known to transmit the disease to other animals. Transmission of PPRV mainly occurs during close contact, inhalation and the virus is shed in nasal and ocular secretions, saliva, urine and feces and also through milk. Fomites such as water, feed troughs and bedding can probably transmit PPRV for a short time, but do not remain

infectious for long periods. The incubation period is 4–6 days, but may range between 3 and 10 days; in most cases, clinical signs appear in 2-6 days.

### 13.1 Epidemiology of outbreak in Bhutan

This is the first recorded outbreak of PPR in Bhutan and it occurred in the Tsheddar goats (religiously rescued goats from slaughter as per the religious belief) confined in the dog pound at Chukha Hydro-Power Corporation Limited project at Chukha on 15th June 2010. 43 goats out of 87 died during the period with a mortality rate of 49 % and morbidity rate of 100 %.

Since the impounding of goats in 2006-07, a total of 83 goats were impounded as Tsheddar by CHPCL, Chukha out of which 43 died with signs of pneumonia. The introduction of 4 goats on 13.4.2010 from Tsimalakha and 5 nos. of goat from P/ling by CHPCL team on 1.6.2010 are believed to be the main cause of the outbreak although the change in weather condition cannot be ruled out as other possible factor. No further investigation has been conducted to prove the possible exposure of P/ling goats with goats across the border in India, where PPR is endemic in the goat population. However, the investigation findings do indicate that some of the goats brought as Tsheddar come from across the border in Phuentsholing or Jaigoan (West Bengal) and it clearly indicates that the outbreak might have entered through illegal import of goats into the country.



*Shooting diarrhea with blood specks*



*Muco-purulent nasal discharge and matted eye lids*



*Lesions in the dental pad and gums typical in PPR*

*Figure 13: Lessons and symptoms of PPR*

## **14. Johnes Disease in Bhutan**

DR. R. B. GURUNG, PhD STUDENT, UNIVERSITY OF SYDNEY, AUSTRALIA

Johne's disease (JD) also known as paratuberculosis is a chronic granulomatous enteritis of ruminants mainly cattle and sheep. The disease is also seen in other domestic and wild ruminants. It is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Clinically affected animals show intermittent diarrhoea and progressively wasting condition. It is a production limiting disease resulting into huge economic loss especially in dairy, beef and wool industry worldwide. Recently it has also become the issue of public health concern due to its association with Crohn's disease in human.

### **14.1 Reported cases in Bhutan**

Several sporadic cases of JD were reported from different dzongkhags since 1999 mostly in mithun and mithun cross and almost all cases were confirmed based on gross PM lesion or presence of causative agent in bowl pinch by acid fast staining. The disease was confirmed through laboratory tests by NCAH in 1999.

### **14.2 Risk of spreading JD in Bhutan**

Native cattle population comprises about 82% of entire cattle population in the country and some farmers still follow traditional practice of cattle migration. Similarly, nomadic community in alpine areas practices migration with yaks and thus once in a year native cattle and yaks share common pasture at temperate region but at different time of the year. Presence of infected individual in either yak or native cattle may contaminate the pasture and develop cross infection and migration practices and sharing common grazing ground is a threat to spread of JD.

Stringent conservation policies of wild fauna protect and allow uninterrupted movement of wildlife through all 12 biological corridors of Bhutan. These biological corridors connect all the national park, sanctuary and reserve. Besides many protected species biological corridors serve home to takin, musk deer, wild water buffalo, gaur, blue sheep, barking

deer and mountain goat. Some of these species share grazing ground with cattle and yaks.

#### *Zoonoses*

The gastronomy of Bhutanese culinary where ruminant intestines are liberally used, it may become great interest of public health due to its association to Crohn's disease in human.

### **14.3 Economic significance of JD in Bhutan**

JD is a potential concern for every country including Bhutan. Livestock farming will be severely affected by the consequences of this disease from reduced milk production, increased culling rate and weight loss due to poor feed conversion ability. There will be loss of time and labor for complying regulatory programs. The magnitude of effect will be more pronounced in rural community especially where livestock is the only means of their living. The cost of diagnosis and research will also increase.

In Bhutan, slaughter of infected animals will be impossible and the animal will carry disease to terminal stage with sufferings throughout thus compromising the animal welfare too. Visiting national parks through ecotourism in national parks will be hampered by the threat of cross infection between wild fauna and surrounding domestic livestock.

### **14.4 What we can do to control JD in Bhutan**

Policy on import of breeding stocks especially in regards to Jersey and mithun may be worthwhile revisiting in regard to ascertaining authenticity of JD free status at source.

Animal health system needs to build international standard diagnostic capacity for JD and a strong human resource base has to be developed through short and long term trainings.

National prevalence of JD needs to be established through combined study of epidemiology and laboratory investigation.

Culling and disposal of infected animals should be implemented and the Livestock Act should include legal provision to culling such animals.

The picture below shows Dr. RB Gurung involved in a PhD project with an aim to develop reliable diagnostic test with high sensitivity, ability to detect infection in early stage, short turnaround time, cost effective and convenient application in JD.



*Figure 14: Protein extraction and assay*



*Figure 15: Animal model infection trial*

---

### **15. Field Application of FMDV-NS ELISA to Detect Antibodies against Non-Structural Proteins of Foot and Mouth Disease Virus in Bhutan**

**DR. JAMBAY DORJI, MASTERS STUDENT, CHAINGMAI UNIVERSITY**

Foot and Mouth Disease (FMD) is a highly contagious viral disease which affects all cloven-hoofed animals and is widespread in many parts of the world. There are seven distinct serotypes and serotypes O, A, C and Asia1 have been confirmed in animals in Bhutan. However, lately only serotypes O and Asia1 are involved in outbreaks since 1998 in the country.

FMD vaccines available in the current markets are highly purified and inactivated. The vaccinated animals induce production of antibodies principally to the structural proteins of the viruses, whereas the infected animals produce antibodies to both the structural and non-structural proteins. Therefore sero-conversion of susceptible animals to non-



structural proteins (NSP) is considered indicative of FMDV infection. The conventional serological diagnosis of FMD using the virus neutralization assay or ELISA relies on the detection of antibodies to the structural proteins of the virus and does not distinguish between vaccinated and infected animals. Assays demonstrating antibodies against NSPs have the potential to differentiate infected from vaccinated animals.

In this study we describe about the use of the non-structural protein 3ABC as antigen in blocking ELISA for antibody detection in cattle following vaccination and/or infection with FMD virus in Bhutan.

The main objectives of the this study are to determine the test ability to differentiate between infected from vaccinated animals; to assess the field applicability of the CEDITEST®FMDV-NS ELISA kit for detection of antibodies against NSP of FMD virus in Bhutan by assessing its sensitivity and specificity; to determine the earliest and the longest periods for 3 ABC antibodies detection in FMD infected animals; and to distinguish the FMD infected/convalescent animals from vaccinated animals for monitoring virus presence or circulation in the study area.

### **15.1 Study area and sources of samples**

A total of 179 serum samples were collected from cattle irrespective of breed, age and sex from Trashigang, Samdrupjongkhar, Chukha and Zhemgang districts and National Nublang Breeding Farm (NNBF), Tashiyangphu in Trashigang. We designed our study into three groups and collected the samples accordingly - sera samples from non-vaccinated and naïve cattle; sera samples from FMD vaccinated cattle at various post vaccination period and sera samples from FMDV infected cattle at various post infection periods.

### **15.2 Laboratory test**

All 179 sera were subjected to antibody detection testing using CEDITEST®FMDV-NS ELISA as per the test protocols of the kit. The optical density (OD) was measured at 450 nm using the ELISA plate reader and

the mean Percentage Inhibition (PI) of each sample was calculated in Microsoft excel.

### 15.3 Results

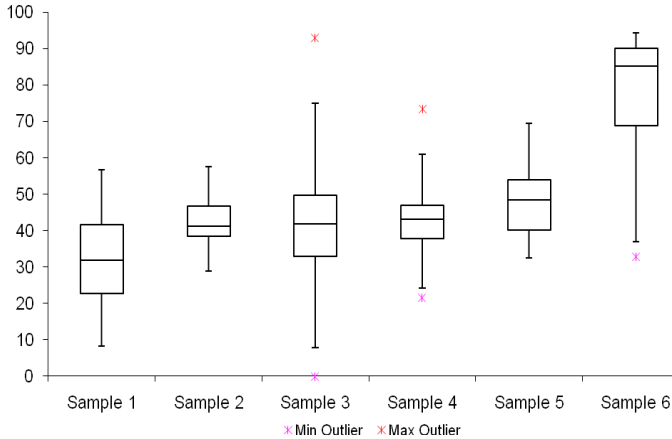


Figure 16: shows the box and whisker plot to graphically depict the summary statistics (median, first and third quartiles, inter-quartile range, outliers, skewness and the tails of the distribution) of the PI values for the various sera samples tested for antibodies against 3 ABC proteins.

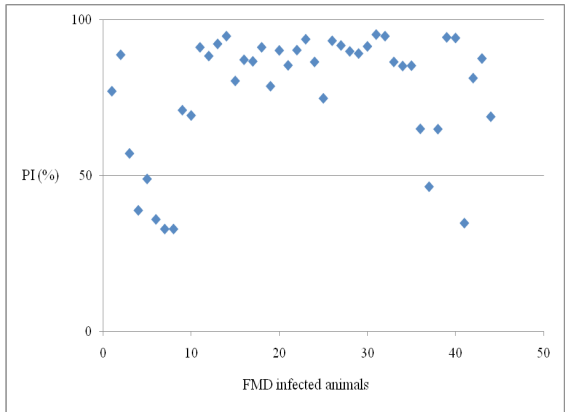


Figure 17: shows the percentage inhibition in FMDV infected animal samples.

Overall, 20.2 % of the serum samples from non-vaccinated/naïve and FMD vaccinated animals had PI > 50 %, indicating positive antibodies against FMDV 3 ABC proteins. The result implies that the sampled animals were exposed to FMDV previously. Since we did not collect the history of the source of animals, it is probable that the animals might have been exposed to FMDV in their previous farms or village.

The sensitivity of 3ABC ELISA for cattle infected with FMDV was 100 % in sera collected and tested 2 to 8.4 weeks FMD post infection. The sensitivity was 50 % in sera collected and tested 5 days post infection and 66.7 % in sera collected and tested 382 days post infection. The specificity for 3ABC ELISA was calculated for the non-vaccinated/naïve animal group only and the specificity were found to be 84.1 %.

The earliest antibody response against the NSP (3ABC) in cattle was measured at day 5 post infection (5 out of 10 samples) in this study. The longest period for antibody detection was 382 days post infection (4 out of 6 samples) in this study.

The practical use of CEDITEST®FMDV-NS ELISA is immense and in Bhutan, perhaps its major use at present may be to serve as a rapid diagnostic kit to confirm FMDV infections in all susceptible animals. It can be used to differentiate infection from vaccination as part of sero-monitoring and surveillance in the field at the national or regional level.



## **16. National Dog Population Management and Rabies Control Programme**

DR. MANOHAR SHARMA, PROJECT COORDINATOR, HSI-DOIL, DOG POPULATION AND RABIES CONTROL; PROJECT

### ***Towards rabies free Bhutan***

Bhutan has been facing problems of increasing dog population and frequent incidences of rabies in dogs, cattle and man. Controlling dog population has always been a challenge. Various methods of control

have been applied in the past, such as mass sterilization, translocation, poisoning and or euthanasia (humane killing) and recently impounding. The later is mainly to provide shelter to the roaming or stray dogs. Despite all these efforts dog population has always been increasing mainly in the towns and cities. In an attempt to address the increasing number of street dogs the Royal Government of Bhutan (RGOB) invited the Humane Society International (HSI), an NGO working for the welfare of animals based in the USA.

Under the aegis of the HSI and RGOB a project entitled National Dog Population Management and Rabies Control Programme (NDPM & RCP) was launched in September 2009. The main aims of the project are (i) to reduce the roaming dog population of Bhutan in a humane and sustainable manner; (ii) To reduce the incidence of rabies and other zoonotic diseases acquired from dogs and dog-bite injuries and (iii) To address community concerns and improve the tourist experience in Bhutan and (iv) To reduce Ministry of Health expenditure in managing rabies and other health issues; pre and post-bite vaccination of humans.

The NDPM & RCP was launched in Bumthang in September 2009. A team of veterinarians and paravets from HSI India and local staff from Department of Livestock joined together and started the programme. The sterilization programme is also called CNVR (Catch Neuter Vaccinate and Release) programme. In this approach dogs are caught in their locations, brought to the field clinics, vaccinated, sterilized, treated with antibiotics, anthelmintics and vitamin B complex then released back to the same location. With CNVR approach the dogs are taken back to their own habitat so that they can live in their own area as long as they live but cannot breed thus the population growth is controlled.

So far the programme has been completed in Bumthang, Thimphu, Punakha and Paro and is ongoing in Sarpang, Wangduephodrang and Chukha dzongkhags. The programme is gradually moving towards rabies endemic dzongkhags. The next priority districts are Samtse and Samdrupjongkhar, to be followed by other Dzongkhags. This way it is planned to cover all the twenty dzongkhags. The target set is to operate 70% of the total national



attempt initiate formal institutional linkage with both livestock and human health facilities located across border focusing primarily on long term disease information exchange between Bhutan and the adjoining Indian States of West Bengal.



*Figure 19: Flash Back of the visit to West Bengal.*

### **17.2 Awareness on rabies for Medical and livestock officials**

Awareness on rabies was conducted at Punakha by MoH for both Medical and Livestock officials from 15th to 16th Oct 09. A vet from NCAH represented the DoL for the awareness program. 30 officials of both medical and livestock were present from central and western region. They were basically made aware on the disease status in the country in human and animals, diagnosis and policy guidelines for prevention and control programs.

### **17.3 Teaching on HSI protocol in dog sterilization for CNR block week**

During the period of 12th to 17th of October 2009, the block week of CNR students were attended by a vet from NCAH. The students of CNR were taught on the protocol of Human society international (HSI). The students were taught on the safe method of anesthesia, aseptic precautions to be followed and effective dog sterilization. During the period 195 dogs were sterilized in total which comprised of Pet dogs: 89 male, 52 female and stray: 30 male, 24 female. About 170 dogs were also vaccinated against rabies.

## 17.4 Dog sterilization program at Serbithang and Babesa

Two days dog sterilization campaign was conducted on 10th and 11th of September 09 by Clinical services section, NCAH for Serbithang and Babesa localities. Considering the increase in stray dog population in these areas, the program was initiated. The team comprised of Vet, technicians and trainees.

In total of 26 dogs (19 females and 11 males) were sterilized and 18 nos. of dogs were vaccinated against rabies. Among them 6 dogs caught were found to be already castrated.

Besides sterilization, the dogs were sampled for parasitic infections.



*Figure 20: Flash back of the field training.*

## 17.5 Creation of Drug Vaccine & Equipment Unit (DVEU)

The Essential Veterinary Drug Program (EVDP) has been renamed as Drugs, Vaccine & Equipment Unit (DVEU) which is a new functional unit under National Centre for Animal Health (NCAH), Serbithang. DVEU has separate mandates and has status on par with Drugs, Vaccine & Equipment Division (DVED) of Ministry of Health. A separate National Veterinary Drug Committee (NVDC) has been formed who will facilitate the updating of essential veterinary drug lists and providing the technical guidelines to DVEU as and when required.

The centralized budget is maintained and operated by the DVEU at NCAH with the technical guidance from the NVDC. DVEU shall prepare its own annual work plan & budget projections based on the actual requisition in

the field by starting separate LC account. From the current year the fund provided for drugs and vaccines to respective Dzongkhags and central farms has been transferred to NCAH. All the animal health facilities and central units in the country should submit six monthly drug reports as per the format provided.

### **17.6 Rapid diagnosis of Rabies using RICT kits in collaboration with Oita University, Japan**

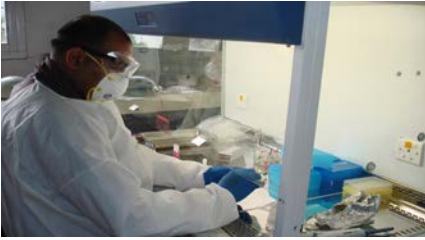
Dr. Kamruddin Ahmed, Associate Professor, Oita University Japan was at the National Centre for Animal Health in the first week of June 2010. The visit by the professor was the result of collaboration between National Centre for Animal Health, Serbithang and the Oita University, Japan on rabies. The main focus was on use Rapid Immunochromatographic test (RICT) for diagnosis of rabies. One of the major outcomes of his visit to our centre was training of field colleagues in RICT.

Laboratory colleagues from Regional Livestock Development Centres and Satellite Veterinary Laboratories, staff from National Centre for Animal Health were trained in appropriate use of RICT kits in the field for diagnosis of rabies. Staff from Regional Livestock Development Centres Wang due, Tshimasham and Satellite Veterinary Laboratory Phuentsholing and NCAH participated in the training program. The other major outcome of the program was optimization of RICT kits for use under our field conditions. Separate protocol for laboratory and field was developed and is being circulated to the concerned after the training program.

The joint collaboration between NCAH and Oita University is foreseen to facilitate establishment of real time PCR diagnosis at our centre at the earliest possible. The gene sequencing of rabies virus originating in Bhutan is under way in Oita University. Once completed, we will have the primers in place to perform PCR diagnosis. During the associate professor's visit, he presented the over view of areas of possible collaboration between Oita University and National Centre for Animal Health. We have agreed to further extend this collaboration. Discussions are underway and we are of the opinion that emerging zoonoses could be one major area for future



collaboration.



*Figure 21: Flash back of the Rapid diagnosis of Rabies*

### **17.7 Crisis Management Centre – Animal Health assisted PCR training at National Centre for Animal Health**

Subsequent to the FAO rapid assessment for the prevention and control of HPAI in Bhutan by David Hadrill, Disease Control Expert, Crisis Management Centre Animal-Health (CMC-AH) of Food and Agricultural Organization (FAO), Dr. Ken Inui, Laboratory expert on molecular diagnosis of Highly Pathogenic Avian Influenza utilizing PCR system was deputed to National Centre for Animal Health, Bhutan w.e.f. 16th to 23rd June 2010.

Dr. Ken Inui, FAO laboratory expert currently working in Vietnam assisted National Centre for Animal Health in setting up and training 4 laboratory officials in real time PCR system for a period of one week. He also gave presentations on essentials of real time PCR system, Porcine Respiratory and Reproductive Syndrome to the laboratory officials at NCAH, Serbithang. The main emphasis was on training on the use of real-time PCR for the diagnosis of HPAI H5N1 and its differential diagnosis. The following were the main areas covered during the training period:

1. Laboratory set-up and work flow and guided us in improving laboratory safety and bio-security
2. Laboratory work, demonstration and practice.
3. Internal quality control, proficiency testing
4. Quality assurance
5. Efficiency testing of the primers probes

6. Preparation of PCR reagents especially the dilutions for primers, probes, controls etc and setting up PCR reactions and optimization of the PCR protocol.
7. Use of iQ5 software for protocol set up, result analysis and interpretation (both for SYBR green and Taqman).
8. Advise/sourcing of optimal reagents, primers for use under

Apart from above, the laboratory expert trained the officials in adapting RNase free practice, RNA extraction using different methodology, aliquot and storage techniques for reagents. Taqman qRT-PCR, Taqman qPCR and SYBR green qPCR techniques were practised for real time PCR diagnosis. With the assistance from CMC-AH, and with the generous help from laboratory expert, FAO who also facilitated us with primers and probes, the National Centre for Animal Health can now perform diagnosis of following disease using real time PCR at Serbithang.

- i. Poultry – Highly Pathogenic Avian Influenza including Influenza A H1N1 (novel virus 2009), Newcastle disease, Infectious Bursal disease, Duck Enteritis virus, Chicken Anemia virus
- ii. Swine – PRRS, Classical swine fever, porcine epidemic diarrhea, Transmissible gastroenteritis, Porcine circovirus
- iii. Bovine – Foot and mouth disease.



*Figure 22: Flash back of the RT-PCR training by FAO expert.*

## **17.8 Veterinary Laboratory Technology Diploma Course at NCAH**

The Veterinary Laboratory Technology Diploma Course held at NCAH, Serbithang in collaboration with the College of Natural Resources, Lobeysa is going on in full swing. The two years long training programme trains twenty eight trainees, out of whom twenty are in-service candidates

and eight, are pre-service candidates.



*Figure 23: Group photo of the trainees with the Chief Guest and other guests during the inauguration of the training program*

The Laboratory Assistants /in-service candidates undergoing the up gradation training programme are Class X passed with a one year certificate course in laboratory technology plus four to five years of working experience in the laboratory and the pre-service candidates are class XII passed.

This up-gradation training program is formally endorsed by the Institute Academic Board of the College of Natural Resources with the consent from the RUB. As the award will be an institute award, the college enjoys the full authority to run the course in line with the wheel of the academic law of the Royal University of Bhutan (RUB).

The curriculum is in the form of Modules spread over a period of 4 semesters, the second year will be entirely a field attachment program where as the first 2 semesters will be a mix of theory and class room teaching at the NCAH, Serbithang. They will be having the second semester examination beginning 30.06.2010 and will break for a short break, which will end their first year of training. During their second year, they will be attached to various livestock centre's to gain field experience so as to enable to work independently in Dzongkhag Veterinary Laboratories after the completion of the diploma course and this fulfills our objective in developing required diagnostic capacity within the animal health service, in achieving required manpower level for all the in-country veterinary laboratories to be able to expand Dzongkhag veterinary laboratories as planned in the 10th five year plan, to provide diagnostic support and disease investigation services to the various Dzongkhag, central farms, private entrepreneurs, backyard farms and to individual farmers and to strengthen overall veterinary laboratory services in the country.