





REPORT

"Training cum Workshop on Biosafety for handling and diagnosis of high risk animal pathogens in ABSL-3 and BSL-3 laboratories"

Conducted for:

'National Network of BSL3/4 Laboratories'

Funded under:

National One Health Mission (NOHM) component of PM-ABHIM

Coordinated by:

Office of the Principal Scientific Advisor, GOI
Indian Council of Medical Research (ICMR), DHR, GOI

Duration:

19th - 23rd August 2024 and 26th - 30th August 2024

Organized by:

ICAR-National Institute of High Security Animal Diseases, Bhopal





BACKGROUND AND CONCEPTION

The significance of biosafety and biosecurity in disease diagnosis has been profoundly realized since the COVID-19 pandemic. With 75% of emerging pathogens being zoonotic, sophisticated and streamlined BSL3/BSL4 lab facilities along with trained manpower are the need of the hour. In response, the Government of India, through the National One Health Mission (NOHM), prioritized capacity building and the harmonization of protocols for outbreak investigation and pandemic preparedness across BSL3 laboratories nationwide. This initiative aimed to establish a cohesive network of BSL3 laboratories capable of coordinated action during emergencies.

Key stakeholders in this initiative included the Indian Council of Medical Research (ICMR), Indian Council of Agricultural Research (ICAR), Department of Biotechnology (DBT), Department of Health Services, Department of Animal Husbandry and Dairying (DAHD) Council of Scientific & Industrial Research (CSIR) etc. These entities needed to collaborate to ensure that both human and animal health sectors could effectively perform their critical roles.

The investigation of zoonotic diseases confronts unique challenges in sampling, sample handling, processing, testing, and reporting, which differs significantly from those encountered in human disease investigations. To ensure the coherent operation of BSL3 labs during crises, it was essential to facilitate knowledge exchange among investigators across various sectors.

As presently the BSL3/4 laboratories adhered to protocols tailored to their specific needs and resources, the training of scientists of these laboratories in adopting standardized practices and procedures in accordance with internationally accepted biosafety guidelines is crucial. This approach would enable network partners to function as a unified, efficient unit during national emergencies and provide insights into biosafety challenges and practical solutions for handling zoonotic and high-risk pathogens.

To harmonize the work culture of BSL3 labs within the network and strengthen national capacity for future pandemics, it was imperative that personnel in these labs are well-trained in general biosafety compliance procedures. Consequently, a training program titled "Training cum Workshop on Biosafety for handling and diagnosis of high risk animal pathogens in ABSL-3 and BSL-3 laboratories" was proposed. This program targeted BSL3 laboratory staff, including scientists, technicians, and support staff.

The training aimed to enhance biosafety and biosecurity knowledge, raise awareness about animal disease diagnostic techniques, and foster a culture of responsibility and mutual collaboration across the different sectors. Additionally, it sought to improve inter-organizational coordination among the BSL3 labs under the network, enabling them to share workloads during emergencies. The training program was designed to ensure both personal and environmental safety from the dangerous infectious pathogens handled by these laboratories.

OBJECTIVES

- 1. Sensitization of participants towards Animal Biosafety Level 3 laboratories design, establishment and maintenance.
- 2. Capacity building for animal experimentation, collection and processing of animal samples with high risk pathogen and their disposal following proper ABSL3 and BSL3 standards.
- 3. Capacity development of participants on basic animal diseases diagnostic techniques and work practices.

PARTICIPATING INSTITUTES AND PARTICIPANTS DETAILS

The details of participants and their affiliations are summarized in the table below:

Sr No	Name of the Participant	Institute	Designation					
ВАТСН	BATCH-1							
1	Dr Riyesh T	ICAR-NRCE, Hisar	Senior Scientist					
2	Dr. Nandu TG	InStem, Bangalore	Project Manager					
3	Dr Alok Kumar Yadav	CCS, NIAH, Baghpat (DAHD)	Assistant Commissioner					
4	Mr. Atul Sirsat	ICMR-NITVAR, Pune	Technician-2					
5	Mr. Sanjai. D RGCB TVM	DBT-RGCB, Kerala	Senior Chief Manager					
6	Dr Subhabrata Sarkar	PGIMER, Chandigarh	Scientist-C					
7	Dr. Himanshu Kaushal	ICMR-NIV, Pune	Scientist D					
8	Dr. Lakshya Veer Singh	DBT-ICGEB , Delhi	Research Scientist					
9	Dr. Arun T R	ICMR- NIV Pune	Scientist B					
BATCH-2								
1	Dr Narayan R	JIPMER, Puducherry	Scientist C					
2	Dr. Aniruddha Jakharia	ICMR-RMRC, Dibrugarh	Scientist-D					
3	Mr. Jijo Koshy	ICMR-NIV, Kerala Unit	Sr. Grade Technician I					
4	Dr. Bharti Bhatia	DBT-THSTI, Faridabad	Principal Scientist - I					
5	Dr. Sarita Jena	BRIC-ILS, Bhubaneswar	Scientist F					
6	Krishan Gopal Thakur	CSIR-IMTECH, Chandigarh	Principal Scientist					
7	Amit Kumar	CSIR-CCMB, Hyderabad	Technical Officer					
8	Dr Faslu Rahman A.T.	ICAR-IVRI, CADRAD, Izatnagar	Senior Technical Officer					
9	Mr. Rizwan Ahmed Ansari	AIIMS, Jodhpur	Technical Assistant					
10	Ravishankar Singh	r Singh Mobile BSL3, Gorakhpur Senior Te						
11	Dr. Shyam Singh Dahiya	ICAR-NIFMD, Bhubaneshwar	Senior Scientist					
12	Dr Shashwati Nema	AIIMS, Bhopal	Addl. Professor					

CONTENT AND SCHEDULE OF TRAINING PROGRAM

	TRAINING cum Workshop SCHEDULE				
	Title		Instructors	Time	
	Day 1	•	<u> </u>		
1	Registration, Pre-training assessment & Interaction with Coordinators	0	Coordinators	9.00-9.30	
2	Biosafety Orientation: Regulations for visitors to NIHSAD bio containment laboratory	0	Dr HV Murugkar	9.30-10.00	
3	Inaugural Function		All NIHSAD Staff	10.00-11.00	
4	Emerging, exotic and transboundry animal diseases: Role of NIHSAD in its control.	L	Dr A Sanyal, Director	11.00-12.00	
5	Role of BSL3 Laboratories network for pandemic preparedness and Sensitization to SOPs for Mock Drill Exercise.	L	Dr Nivedita Gupta	12.00-1.00	
6	Visit to Animal Receiving Shed, Animal Holding Shed, SPF Unit and Supporting Facilities	V	Dr Manoj Kumar	2.00-3.00	
7	Trapping and Sampling of Birds, Bats, Rodents etc. under field conditions for zoonotic disease surveillance.	L	Dr Satish Pande	3.00-4.00	
8	Demonstration of setting up of traps and nets for catching animals, birds and vectors for sample collection.	Р	Dr Satish Pande & ELA foundation team	4.00-5.30	
	Day 2				
9	Visit to Slaughter House, State Animal Disease Investigation laboratories, Veterinary Polyclinic and State Animal Farm.	V	Dr Fateh Singh & Dr Ashwin Raut	7.00-12.00	
10	Recommended practices for advancing biosafety while working in animal biosafety level-3 [ABLS-3] laboratory.	L	Dr Ashwin Raut	12.00-1.00	
11	Entry inside the ABSL3 Laboratory and Orientation of ABSL3	Р	Dr Venkatesh	2.00-300	
12	Orientation on Animals Isolators (HEPA Filtered Gloveboxes/Class III cabinets) Cleaning, Decontamination & Setup for Animal Experiments.	0	Dr Ashwin Raut & 3.00-5.30 Dr Manoj Kumar		
13	Experimental infection of animals with highrisk pathogens in Animal Isolators.	Р			
14	Collection of samples from infected animals in Animal Isolators and transport to BSL3 Lab.	Р			
	Day 3	•			
15	Handling and Collection of samples from infected animals in ABSL3 containment rooms	P	Dr Rajukumar &	9.00-12.00	
16	Postmortem of animals and collection of samples in ABSL3 Postmortem rooms	P	Dr Senthil		
17	Indian initiatives for Future Pandemic Preparedness	L	Dr Sindura Ganapati	12.00-1.00	
19	Postmortem of animals and collection of samples in Biosafety cabinets in BSL3.	Р	Dr Manoj & Dr Anubha	2.00-3.00	
20	Processing of Animal and Vector samples for NAATs and Virus Isolation.	Р	Dr Nagarajan, Dr	3.00-4.00	
21	Virus isolation in embryonated eggs & laboratory animals & harvesting of allantoic fluids.	P	Tosh & Dr Pradeep	4.00-5.30	
	Day 4	1.	T	T	
22	One Health and Zoonosis	L	Dr SB Barbudhe	9.00-9.45	
22	Concerns and measures for minimizing abattoir associated Highriks Zoonosis	L	Dr S. Chaudhari	9.00-9.45	
23	Outbreak Investigation & Pathogen Discovery using Next Generation Sequencing.	L	Dr Anamika Mishra	9.45-10.30	
24	Biowaste management, BSC Validation, HEPA Filter Replacement etc.	P	Dr HV Murugkar	11.00-1.00	
25	Lumpy Skin Disease (LSD) diagnosis by virus titration and neutralization assay.	Р	Dr N Mishra & Dr Sudhakar & Dr Kalai	2.00-3.30	
26	African Swine Fever virus isolation in cell cultures & its confirmation by haemadsorbtion.	P	Dr Fateh Singh & Dr Senthil Kumar	4.00-5.30	
	Day 5				
27	Differential diagnosis of low pathogenic (H9) and highly pathogenic (H5) Type A influenza infection in chicken by multiplex RTqPCR.	Р	Dr Atul Pateriya & Dr Anamika	9.00-11.00	
28	Haemagglutination/Haemagglutination inhibition assay & Serological detection of Avian Influenza virus antibodies by in-house ELISA.	Р	Dr S Bhatia & Dr Naveen	11.00-1.00	
29	Post Training Assessment & Interaction with faculty	0	Coordinators	2.00-3.00	
30	Valedictory Function		All NIHSAD Staff	3.00-4.00	

DAYWISE DETAILED OVERVIEW OF 5-DAY TRAINING PROGRAM:

Training Schedule Day-1

The Biosafety Orientation of the participants, led by Dr. HV Murugkar, Principal Scientist, Biosafety Officer and Course Coordinator of the training program was the first activity of the training which covered training of the participants in essential biosafety regulations.

The inaugural function involved all NIHSAD staff, setting the stage for the event. Dr. A Sanyal, Director ICAR-NIHSAD, served as the Course Director of the training, he discussed the role of ICAR-NIHSAD in pandemic preparedness using advanced biosafety facilities. Dr. Ashwin A. Raut Principal Scientist, Group leader of Zoonosis Disease Group (ZDG) and the Course Coordinator of the training program presented the outline of the five-day training program. Dr Anamika Mishra, Principal Scientist, and the training course co-coordinator presented the vote of thanks. The inaugural event was coordinated by Dr Anubha Pathak, Scientist and training course co-coordinator.

Dr A Sanyal delivered a lecture on 'Emerging, exotic and transboundry animal diseases: Role of NIHSAD in its control' to the participants to elaborately describe the strategies employed by ICAR-NIHSAD to monitor, prevent, and control the spread of diseases in the country.

The first guest lecture was delivered by Dr. Nivedita Gupta, Scientist G and Chief of Communicable Diseases at the Indian Council of Medical Research (ICMR), New Delhi. Dr. Gupta underscored the critical role of the Biosafety Level 3 (BSL-3) laboratory network in enhancing pandemic preparedness. She urged the trainees to actively participate in the formulation of Standard Operating Procedures (SOPs) pertinent to their respective laboratories and share them for streamlining the SOPs followed throughout the country.

Dr. Manoj Kumar, Senior Scientist and Training Course Co-coordinator guided a visit to the Animal Receiving Shed, Animal Holding Shed, SPF Unit, and supporting facilities.

Dr. Satish Pande, Director, Ela Foundation and OENSL, Pune delivered guest lectures and conducted live demonstrations on trapping and sampling of birds, bats, rodents, and other animals using bat mist nets, Sherman live traps, snap traps, baited traps, harp traps etc. under field conditions for zoonotic disease surveillance. This helped the participants to develop a through idea about the techniques and issues of animal trapping for disease surveillance. It is anticipated to enhance cross sector participation in Joint outbreak investigation at times of emergencies.

Training Schedule Day-2

The visit to the Slaughterhouse, State Animal Disease Investigation Laboratory, Veterinary Polyclinic, and State Animal Farm was led by Dr. Ashwin Raut and Dr. Fateh Singh. A lecture providing insights on recommended practices for advancing biosafety while working in an animal biosafety level-3 (ABLS-3) laboratory was delivered by Dr. Ashwin A Raut. These visits aided in understanding and practical observation of different human animal interfaces. This also prepared the participants of human health sector to enlist and visualize the possible zoonotic disease transmission opportunities.

Dr. G. Venkatesh facilitated the entry of the participants inside the ABSL3 Laboratory and provided an orientation. Dr. Ashwin Raut and Dr. Manoj Kumar conducted an orientation on the decontamination and setting up of Animal Isolators (HEPA Filtered Gloveboxes/Class III cabinets) for animal experiments. The program also included sessions on the experimental infection of animals with high-risk pathogens in Animal Isolators, as well as the collection of samples from infected animals and their transport to the BSL3 Lab.

[Ma1] Training Schedule Day-3

Handling and collection of samples from infected animals in ABSL3 containment rooms were overseen by Dr. K. Rajukumar and Dr. D. Senthil. The procedure of postmortem of animals and the collection of samples in ABSL3 postmortem rooms were overviewed.

Dr. Sindura Ganapati, Vising PSA Fellow, Office of Principal Scientific Advisor, GOI has delivered the guest lecture on 'Indian initiatives for future pandemic preparedness' elaborating the country's capacity to handle potential outbreaks.

Postmortem of animals and the collection of samples in biosafety cabinets in BSL3 while using PAPRs were demonstrated by Dr. Manoj Kumar. Processing of animal and vector samples for nucleic acid amplification tests (NAATs) and virus isolation was carried out by Dr. S. Nagarajan, Dr. C. Tosh, and Dr. Pradeep N Gandhale. Additionally, virus isolation by inoculation of embryonated chicken eggs and laboratory animals, along with the harvesting of allantoic fluids, was demonstrated.

Training Schedule Day-4

Concerns and measures for minimizing abattoir-associated high-risk zoonosis were addressed by Dr. S. Chaudhari Professor & Head, Department of Veterinary Public Health and Epidemiology, MAFSU, Nagpur in the form of a guest lecture, focusing on strategies to reduce the transmission of diseases from animals to humans. An online lecture on "Zoonosis and One health" was delivered by DR SB Barbudhe, Director ICAR- NRCM, Hyderabad.

The lecture on Outbreak investigation and pathogen discovery using next-generation sequencing was delivered by Dr. Anamika Mishra, she elaborated on the use of advanced genomic techniques to identify and track pathogens. Dr. HV Murugkar conducted the session on Biowaste management, biosafety cabinet (BSC) validation, and HEPA filter replacement, ensuring that participants can be trained to maintain a safe and compliant BSL3/4 laboratory environment. The technique of virus titration and neutralization of viral disease diagnosis was elaborately demonstrated in BSL3 laboratory, using Lumpy Skin Disease virus (LSDV) model by Dr. N. Mishra, Dr. Sudhakar, and Dr. Kalayarasu. Additionally, Dr. Fateh Singh and Dr. D Senthil Kumar demonstrated virus isolation in cell cultures and confirmation by Haemadsorption techniques with African Swine Fever Virus model.

Training Schedule Day-5

On the final day of the training program, the differential diagnosis of low pathogenic (H9) and highly pathogenic (H5) Type A influenza infection in chickens was demonstrated using multiplex RT-qPCR by Dr. Atul Kumar Pateriya. The final session on Haemagglutination (HA) and Haemagglutination

Inhibition (HI) assays, along with serological detection of Avian Influenza virus antibodies using inhouse ELISA, were conducted by Dr. S. Bhatia and Dr. Naveen Scientist. Post-training assessment and interaction with faculty were coordinated by the designated coordinators, ensuring comprehensive feedback and learning. The valedictory function, involving all NIHSAD staff, marked the conclusion of the training program, celebrating the achievements and efforts of all participants by distribution of certificates.





Training cum Workshop on Biosafety for handling and diagnosis of High Risk Animal Pathogens in ABSL-3 and BSL-3 Laboratories under National One Health Mission at ICAR-NIHSAD, Bhopal (Batch-2, 26th to 30th August, 2024)

FEEDBACK OF THE PARTICIPANTS

The feedback provided by the participants has been summarized in the table below:

		BATCH -I					BATCH -II				
S.		From:19 Aug, 2024 to 23 Aug					From: 26 Aug, 2024 to 30 Aug, 2024				
No	Area	(Total = 9 Trainees)					(Total = 12 Trainees)				
		Excellent	Very Good	Good	Average	Bad	Excellent	Very Good	Good	Average	Bad
1	Format of Workshop	7	2	-	-	-	4	6	2	-	-
2	Lectures delivered during the workshop	8	1	-	-	-	3	7	2		
3	Facilities available during the workshop	7	2	-	-	-	4	6	2	-	-
4	Laboratory visit experience	7	2	-	-	-	10	2	-	-	-
5	Practical's and demonstrations	9	-	-	-	-	8	3	1		
6	Boarding and lodging provided	5	4	-	-	-	3	6	1	1	-
7	Do you feel that this workshop served your needs (Yes / No)	Yes all					Yes all				

EXPERIENCE, CHALLENGES AND LESSONS FOR THE FUTURE

The training program that aimed at enhancing national pandemic preparedness cultivated an essential collaborative environment between the human health and veterinary sectors. During the various sessions there were discussions and clarifications about the SOPs and their challenges in their implementations in the different laboratories. The program successfully delivered comprehensive training and expert scientific guidance about animal disease investigations, different types of human animal interfaces and the risks involved. Inter-institutional and interpersonal liasoning has been promoted through these training sessions that are expected to be pillars for strengthening the long term goals under National One Health Mission and Pandemic Preparedness.

Though some guest lectures had to be conducted online as the invited faculty were unable to travel on short notice, the knowledge exchange was valuable for the participants. The participants were equipped with the necessary skills and knowledge to work safely in ABSL-3 and BSL-3/4 laboratories.

With the experience gained from the training program of the first two batches and ample preparation time for the upcoming batches, further training programs are expected to be conducted with excellence. It may be emphasized here that the personnel being nominated by the network laboratories should be carefully selected with intent to participate in disease diagnosis, outbreak investigations and joint outbreak response teams. Constant efforts will be made to sustain the collaborations developed during the training thorough exchange of knowledge and experiences and continuous guidance to the network partners as and when required.

RESOURCE FACULTY FOR THE TRAINING PROGRAM

Resource person	esource person Designation		Contact				
Dr Aniket Sanyal	Director	Course Director	director.nihsad@icar.gov.in				
Dr Harshad Murugkar	Principal Scientist & Biosafety Officer	Course Coordinator	harshad.murugkar@icar.gov.in				
Dr Ashwin Ashok Raut	Principal Scientist & Group Leader, Zoonosis	Course Coordinator	ashwin.raut@icar.gov.in				
Dr Anamika Mishra	Principal Scientist	Course Co-Coordinator	anamika.mishra@icar.gov.in				
Dr Manoj Kumar	Senior Scientist	Course Co-Coordinator	manoj.kumar4@icar.gov.in				
Dr Atul Pateriya	Senior Scientist	Course Co-Coordinator	atul.pateriya@icar.gov.in				
Dr Anubha Pathak	Scientist	Course Co-Coordinator	anubha.pathak@icar.gov.in				
GUEST FACULTY							
Name	Designation	Organization					
Dr Nivedita Gupta	SCIENTIST G & Chief CD	ICMR, New Delhi					
Dr Sindura Ganapathi	Visiting PSA Fellow	Office of the Principal Scientific Advisor, New Delhi					
Dr Satish Pande	Director	Ela Foundation and OENSL, Pune					
Dr SB Barbudhe	Director	ICAR-National Meat Research Institute, Hyderabad					
Dr Sandeep Chaudhari	Professor & Head	Veterinary Public Health	Department, MAFSU, Nagpur				
RESOURCE FACULTY	OF ICAR – NIHSAD						
Name	Designation	Contact Details					
Dr. C. Tosh	. C. Tosh Principal Scientist		Chakradhar.tosh@icar.gov.in				
Dr. Niranjan Mishra	Principal Scientist	Niranjan.mishra1@icar.gov.in					
Dr. Sandeep Bhatia	Principal Scientist	Sandeep.bhatia@icar.gov.in					
Dr. Rajukumar K.	Principal Scientist	K.rajukumar@icar.gov.in					
Dr. S. Nagarajan	Principal Scientist	S.nagarajan@icar.gov.in					
Dr. G. Venkatesh	Principal Scientist	G.venkatesh@icar.gov.in					
Dr. S.B. Sudhakar	Senior Scientist	Sb.sudhakar@icar.gov.in					
Dr. Fateh Singh Senior Scientist		Fateh.singh@icar.gov.in					
Dr. S. Kalaiyarasu	Senior Scientist	Kalaiyarasu.s@icar.gov.in					
Dr. Senthil Kumar D. Senior Scientist		Senthil.d@icar.gov.in					
Dr. Pradeep Gandhale Senior Scientist		Pradeep.gandhale@icar.gov.in					
Dr. Naveen Kumar	Scientist	Naveen.kumar4@icar.gc	ov.in				



Image: Participant introduction with Team ICAR-NIHSAD on the event of inaugural function



Image: Distribution of Training cum Workshop completion Certificates to the Participants



National Joint Outbreak Investigation Team (NJORT)

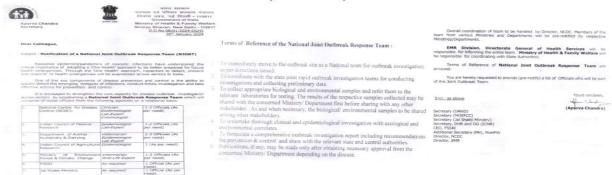


Image: A snippet of online guest lecture delivered by Dr Nivedita Gupta, Chief CD, ICMR, New Delhi



Stakeholders of the National One Health Mission

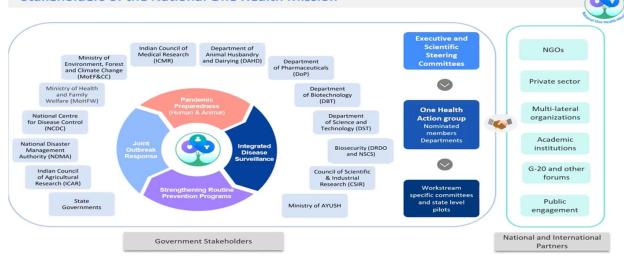


Image: A snippet of online guest lecture delivered by Dr Sindura Ganapati, PSA Fellow, PSA Office, GOI



Image: Participants visit Receiving Shed of ICAR-NIHSAD



Image: Participants dressed up in BSL3 facility of ICAR-NIHSAD



Image: Briefing on ABSL3 facility of NIHSAD by Animal BSO to participants.



Image: Trainees observe experimental infection of birds in Animal Isolators inside ABSL3 facility

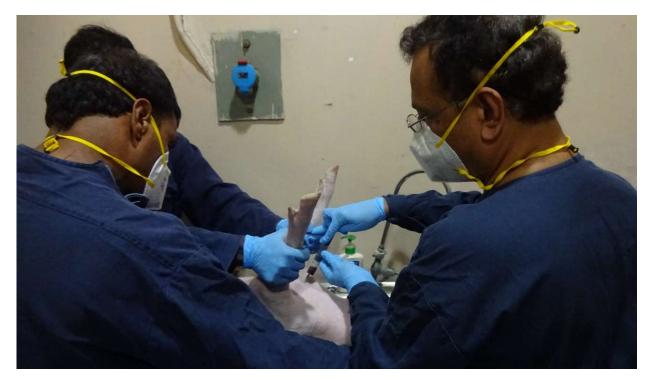


Image: Participants witness collection of blood sample from pig inside ABSL3 Facility



Image: Trainees observe harvesting of allantoic fluid for isolation of Avian Influenza Virus



Image: Participants receive training to wear PAPR inside the BSL3 facility



Image: Participants working wearing PAPR inside the BSL3 facility



Image: Team Ela foundation demonstrates installation and usage of traps to trap wild animals for collection of samples for zoonotic disease surveillance



Image: Team Ela foundation demonstrates trapping techniques for Wild Birds/Bats to trainees



Image: Participants visit slaughterhouse to gain insights on humane slaughter of animals, HACCPs in slaughterhouse and its public health significance



Image: Participants visit stray animal wing at Veterinary Polyclinic



Image: Trainees visit DI lab facility to witness sample collection, processing and diagnosis of veterinary diseases



Image: Trainees visit state cattle farm to witness rearing and milk production facilities