भाकृअनुप – राष्ट्रीय पशुरोग जानपदिक एवं सूचना विज्ञान संस्थान
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Action Plan for PPR surveillance and monitoring under (PPR-EP) CADCP, (LH&DC Scheme), DAHD, GoI

Sampling plan 2

KARNATAKA

Post-vaccination Sampling Plan (Seromonitoring) Year 2023

Sero-monitoring of PPR in Karnataka 2023

PPR/SEROMON/NIVEDI/SP-II_Karnataka16/2023

Stratified Random Sampling:

Species Selected for Stratification = Sheep + Goat

Number of Villages Having 500 + (Sheep + Goat) = 5782

Design Level Prevalence = 0.3

Cluster Level Prevalence = 0.03

Sensitivity of the test used = 0.92

Specificity Level Prevalence = 0.98

Total No of Villages (Clusters) Selected = 108Total

No of Animals to be Sampled = 10 x 108 = 1080

Back to Calculation

VILLAGE_NAME	VILLAGE_S. No. Code	DISTRICT_NAME	BLOCK_Code	BLOCK_NAME	Goats	Sheep	Sheep + Goat	Number of units (animals) to sample	Goat Proportion	Sheep Proportion
Fakeerabudihal	1.	Bagalkot	17	Badami	420	1285	1705	10	2	8
Dammur	2.	Bagalkot	125	Hungund	125	1673	1798	10	1	9
Ilal	3.	Bagalkot	125	Hungund	785	3558	4343	10	2	8
Virapur	4.	Bagalkot	125	Hungund	1182	3543	4725	10	3	7
Chikkanayakanahal	5.	Bengaluru Urban	23	Bangalore East	33	488	521	10	1	9

li										
Doddamaranahalli	6.	Bengaluru Urban	25	Bangalore South	317	294	611	10	5	5
Guddadahalli	7.	Bengaluru Urban	24	Bangalore North	338	366	704	10	5	5
Mandur	8.	Bengaluru Urban	23	Bangalore East	427	318	745	10	6	4
Bangalore (M Corp.) -Ward No.50	9.	Bengaluru Urban	23	Bangalore East	639	621	1260	10	5	5
Shivanapura	10.	Bengaluru Urban	24	Bangalore North	310	1325	1635	10	2	8
Chandapur	11.	Bidar	40	Bhalki	274	587	861	10	3	7
Ghotala	12.	Bidar	30	Basavakalyan	1194	406	1600	10	7	3
Rajeshwar	13.	Bidar	30	Basavakalyan	1454	671	2125	10	7	3
Malakandevarahatti	14.	Bijapur	45	Bijapur	1076	605	1681	10	6	4
Mulawad	15.	Bijapur	31	Basavana Bagevadi	1407	1313	2720	10	5	5
Dyaberi	16.	Bijapur	45	Bijapur	2533	1863	4396	10	6	4
BAALAGUNASE	17.	Chamarajanagar	157	Kollegal	405	306	711	10	6	4
Settahalli	18.	Chamarajanagar	157	Kollegal	634	201	835	10	8	2
GAAJANOORU	19.	Chamarajanagar	157	Kollegal	815	199	1014	10	8	2
Kunagalli	20.	Chamarajanagar	157	Kollegal	881	536	1417	10	6	4
Tellanur	21.	Chamarajanagar	157	Kollegal	779	759	1538	10	5	5
Vemagallu	22.	Chikballapur	265	Sidlaghatta	34	511	545	10	1	9
Manchanabele	23.	Chikballapur	59	Chikkaballapura	53	685	738	10	1	9
Marappanahalli	24.	Chikballapur	59	Chikkaballapura	460	398	858	10	5	5
Thimmampalli	25.	Chikballapur	19	Bagepalli	78	973	1051	10	1	9
Kuruburu	26.	Chikballapur	66	Chintamani	246	883	1129	10	2	8
Yalagalahalli	27.	Chikballapur	59	Chikkaballapura	156	1057	1213	10	1	9
Nallaguttapalli	28.	Chikballapur	19	Bagepalli	265	1596	1861	10	1	9
Yellampalli	29.	Chikballapur	19	Bagepalli	288	1644	1932	10	1	9
Gadihalli	30.	Chikmagalur	280	Tarikere	78	603	681	10	1	9
Sindigere	31.	Chikmagalur	61	Chikmagalur	330	553	883	10	4	6
Devanur	32.	Chikmagalur	136	Kadur	13	960	973	10	0	10

Sampling Plan 2

B.T.Mallenahalli	33.	Chikmagalur	136	Kadur	180	953	1133	10	2	8
P.Kodihalli	34.	Chikmagalur	136	Kadur	173	1291	1464	10	1	9
Devagondanahalli	35.	Chikmagalur	61	Chikmagalur	853	686	1539	10	6	4
Kadaba	36.	Dakshin Kannad	230	Puttur	602	0	602	10	10	0
Aithoor	37.	Dakshin Kannad	230	Puttur	696	0	696	10	10	0
Mallapura	38.	Davangere	129	Jagalur	0	1208	1208	10	0	10
Sasvihalli	39.	Davangere	99	Harapanahalli	322	1323	1645	10	2	8
Nagarasanahalli	40.	Davangere	72	Davanagere	440	2067	2507	10	2	8
Sathur	41.	Davangere	99	Harapanahalli	528	2185	2713	10	2	8
Togarikatti	42.	Davangere	99	Harapanahalli	484	5522	6006	10	1	9
Harobelavadi	43.	Dharwad	75	Dharwad	645	651	1296	10	5	5
Hebballi	44.	Dharwad	75	Dharwad	1279	1931	3210	10	4	6
Guddadbudihal	45.	Gadag	211	Mundargi	515	1792	2307	10	2	8
Asuti	46.	Gadag	239	Ron	2095	4184	6279	10	3	7
Ganjalnkhed	47.	Gulbarga	87	Gulbarga	576	0	576	10	10	0
Yetabarpur	48.	Gulbarga	65	Chincholi	655	0	655	10	10	0
D.Ghangapur	49.	Gulbarga	1	Afzalpur	888	0	888	10	10	0
Medak	50.	Gulbarga	251	Sedam	524	377	901	10	6	4
Hongunta	51.	Gulbarga	67	Chitapur	1159	157	1316	10	9	1
Madan Hipperga	52.	Gulbarga	3	Aland	3033	903	3936	10	8	2
Hullangala	53.	Hassan	12	Arkalgud	412	172	584	10	7	3
Valagerehalli	54.	Hassan	57	Channarayapatna	256	439	695	10	4	6
Hullekere	55.	Hassan	13	Arsikere	297	435	732	10	4	6
Rajanasiriyur	56.	Hassan	37	Belur	216	658	874	10	2	8
S.Ankanahalli	57.	Hassan	112	Hole Narsipur	26	853	879	10	0	10
Nerlige	58.	Hassan	13	Arsikere	498	457	955	10	5	5
Thirupathihalli	59.	Hassan	13	Arsikere	655	489	1144	10	6	4
Chikkalkur	60.	Hassan	13	Arsikere	274	1100	1374	10	2	8
Belagumba	61.	Hassan	13	Arsikere	635	779	1414	10	4	6
Mududi	62.	Hassan	13	Arsikere	926	510	1436	10	6	4
Paduvanahalli	63.	Hassan	13	Arsikere	365	1136	1501	10	2	8

Sampling Plan 2

Rampura	64.	Hassan	13	Arsikere	700	4924	5624	10	1	9
Chattra	65.	Haveri	51	Byadgi	349	694	1043	10	3	7
Itagi	66.	Haveri	236	Ranibennur	344	833	1177	10	3	7
Choudayya Danapur	67.	Haveri	236	Ranibennur	334	1275	1609	10	2	8
Kalledevar	68.	Haveri	51	Byadgi	564	1551	2115	10	3	7
Hirelingadahalli	69.	Haveri	105	Haveri	1094	2003	3097	10	4	6
Thalur	70.	Kolar	26	Bangarapet	195	364	559	10	3	7
Byatanur	71.	Kolar	207	Mulbagal	5	601	606	10	0	10
Kornalli	72.	Kolar	276	Srinivaspur	303	490	793	10	4	6
Kuppur	73.	Kolar	192	Malur	59	753	812	10	1	9
Nachagundlahalli	74.	Kolar	207	Mulbagal	174	669	843	10	2	8
Maliyappanahalli	75.	Kolar	156	Kolar	201	806	1007	10	2	8
Nutuve	76.	Kolar	192	Malur	327	690	1017	10	3	7
Sangasandra	77.	Kolar	207	Mulbagal	354	1947	2301	10	2	8
Sangapur	78.	Koppal	160	Koppal	30	1976	2006	10	0	10
Chikmyageri	79.	Koppal	302	Yelbarga	516	3095	3611	10	1	9
Indargi	80.	Koppal	160	Koppal	439	6058	6497	10	1	9
Allapatna	81.	Mandya	263	Shrirangapattana	181	396	577	10	3	7
Palagrahara	82.	Mandya	215	Nagamangala	256	435	691	10	4	6
Ravani	83.	Mandya	189	Malavalli	777	85	862	10	9	1
Anunahalli	84.	Mandya	227	Pandavapura	144	722	866	10	2	8
Belathur	85.	Mandya	184	Maddur	484	423	907	10	5	5
Kaggalipura	86.	Mandya	189	Malavalli	691	717	1408	10	5	5
Chottanahalli	87.	Mandya	189	Malavalli	831	721	1552	10	5	5
Dundanahalli	88.	Mandya	184	Maddur	953	1860	2813	10	3	7
Manuganahalli	89.	Mysore	126	Hunsur	310	235	545	10	6	4
Hadajana	90.	Mysore	213	Mysore	512	139	651	10	8	2
Somanathapura	91.	Mysore	286	Tirumakudal - Narsipur	594	184	778	10	8	2
Udburu	92.	Mysore	213	Mysore	714	245	959	10	7	3

Huyilalu	93.	Mysore	213	Mysore	283	830	1113	10	3	7
Moduru	94.	Mysore	126	Hunsur	394	837	1231	10	3	7
Nerale	95.	Mysore	218	Nanjangud	784	877	1661	10	5	5
Kardigudda	96.	Raichur	73	Devadurga	320	520	840	10	4	6
Gajral	97.	Raichur	232	Raichur	84	1656	1740	10	0	10
Kalmangi	98.	Raichur	267	Sindhnur	100	1961	2061	10	0	10
Rampur	99.	Raichur	182	Lingsugur	668	1642	2310	10	3	7
Bagalwad	100.	Raichur	196	Manvi	0	2521	2521	10	0	10
Chakkere	101.	Ramanagara	56	Channapatna	207	524	731	10	3	7
Hunsanahalli	102.	Ramanagara	144	Kanakapura	403	383	786	10	5	5
Thimmasandra	103.	Ramanagara	56	Channapatna	272	555	827	10	3	7
Shuivanegowdanaddoddi	104.	Ramanagara	144	Kanakapura	912	485	1397	10	7	3
Bilagumba	105.	Ramanagara	233	Ramanagara	543	1886	2429	10	2	8
Gejjenahalli	106.	Shimoga	259	Shimoga	50	481	531	10	1	9
Guledahalli	107.	Shimoga	257	Shikaripura	201	364	565	10	4	6
KATTIGEHALLA	108.	Shimoga	257	Shikaripura	625	8	633	10	10	0
Kaginalli	109.	Shimoga	257	Shikaripura	74	623	697	10	1	9
Anavatti	110.	Shimoga	274	Sorab	461	252	713	10	6	4
Thumrihosuru	111.	Shimoga	257	Shikaripura	64	671	735	10	1	9
Kappanahalli	112.	Shimoga	257	Shikaripura	294	473	767	10	4	6
AGASAVALLI HOSURU	113.	Shimoga	259	Shimoga	704	353	1057	10	7	3
Kekkera (TP) - WardNo.14	114.	Yadgir	151	Kekkera	220	1064	1284	10	2	8
Kadechur	115.	Yadgir	300	Yadgir	1015	4817	5832	10	2	8

Karnataka

Sampling Plan 2

Village/Epi-Unit- Samples collection Sheet

Annexure 1- for sampling plan 2

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Action Plan for PPR surveillance and monitoring under (PPR-EP) CADCP, (LH&DC Scheme), DAHD, GoI

Serum samples from each Epi-unit should contain the following details for sero-monitoring of PPR under PPR-EP programme

Programme : PPR-EP **State:**

Plan : Sampling Plan 2 : **Year: 2023**

Sl.No of Village as per Sampling plan:

Sampling Plan and Purpose	**For eg. Sampling Plan-2 (Postvaccination survey)**
Village/ Epi-unit Name:	
Village/ Epi-unit ID as per sample frame	**S. No.**
GPS Location- N-Latitude:	
GPS Location- E-Latitude:	
Date of Sample collection:	

Geographical data

State Name	
District Name	
Block/Taluk/Tehsil Name	
Village/ Epi-unit Name	
Date of Sample collection:	
Vaccination at epi-unit	**Yes or No if yes please provide the following details**
Date of vaccination (Extract)	
Vaccine (Make):	
Vaccine Batch:	
Name & Contact No. of Veterinarian under whom this village jurisdiction comes for veterinary services	

Sampling Plan 2

Annexure -1 **Model Sheet- ANIMAL DATA- Sheet for each Epi-unit-** Write appropriately or (✓) tick Mark in the relevant column

S. No	Sample ID	Species		Age group	Breed			Sex		Animal Husbandry Practices				Health status	
Village Name : Hosakote,	Starting letter of village name followed by 1 to 10 as the case	Sheep (1)	Goat (2)	6-12 months (1)	Breed Name	Local/ND (1)	Cross breed (2)	Male (1)	Female(2)	Nomadic(1)	Intensive(2)	Semi-Intensive (3)	Extensive (Free range) (4)	Apparently healthy (1)	Diseased (2)
1.	H-1 (eg.)		✓	✓		✓			✓			✓		✓	
2.	H-2														
3.	H-3														
4.	H-4														
5.	H-5														
6.	H-6														
7.	H-7 (eg.)		✓	✓		✓		✓					✓	✓	
8.	H-8														
9.	H-9														
10.	H-10	✓		✓		✓			✓			✓		✓	

Karnataka **Sampling Plan 2**

Instructions/Note:

1. Please refer to the guideline for the collection of samples from epi-units **(Annexure 2)**
2. While collecting the blood samples from the animals, the animal data (Model table sheet provided- **Annexure -1 Above**) compulsory should be collected.
3. Labeling of serum sample vials should be written as Starting letter of village name followed by 1 to 10 as the case may be for the given sample frame for the sampling plan for the given year. For Example- village name Hosakote, serial no 1: then the sample ID will be "H1"
4. Please refer to the guidelines for collection, storage, and transportation of samples **(Annexure 3)**
5. All the serum samples after separation should be stored in the **inner thread screw cap 1.8 or 2 mL serum cryovials** properly without leakage and can be stored at -20 ℃ in the deep freezer.
6. All the labeled serum samples vials (10 Samples) of a particular village should be packed in a single plastic Ziplock pouch and **labelled/Stricker** should be placed over the pouch (Labeled with State, District, Block, and Village name, as per sampling frame) and to be transported to the designated laboratory in the cold chain at 4° C (Ice Pack).

Signature of the Local Veterinarian/ District Deputy Director of

Animal Husbandry and Veterinary Service Department,

Signature of State Nodal Officer for PPR-EP

Please submit the samples to final designated laboratory for testing.

To,

Dr. V. BALAMURUGAN,
Principal Scientist (Veterinary Microbiologist),
Scientist In-Charge-PPR Research Laboratory
(WOAH Reference Laboratory Network for PPR -South India),
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Mobile:+91-9481807438 (WhatsApp);9108427438

Annexure 2

भाकृअनुप - राष्ट्रीय पशुरोग जानपदिक एवं सूचना विज्ञान संस्थान
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Action Plan for PPR surveillance and monitoring under (PPR-EP) CADCP, (LH&DC Scheme), DAHD, GoI

GUIDELINES

The sampling plan for seromonitoring and surveillance of Peste des Petits Ruminants (PPR) under the National PPR Eradication Programme (PPR-EP) has been prepared as per WOAH(OIE)/FAO-Global Control and Eradication Strategy for PPR guidelines (FAO-OIE,2015).

Post-vaccination effectiveness or Assessments:

To assess the immune response to vaccination (vaccine efficacy), post-vaccination evaluation (PVE), the second serosurvey to be carried out using the above random designs, **after days 60 to 90th-day post-vaccination (within 90 days of postvaccination samples collection should be completed).** It is assumed that at least 50% of vaccinated epi-units, will have at least 70% of the seropositive population. **A total of a maximum of 9 to 10 samples from each of the selected villages / epi-unit to be collected randomly from 6 to 12 months aged animals only,** from three or four selected flocks, with earlier described designed population species proportion ratios in the **provided sampling plan ((No. of samples mentioned in the each of village provided in the sampling frame for each respective State/UT should be as such directly).** Thus, a maximum of **1080 secondary animal units (108 villages x 10 samples) are to be collected for** sero-monitoring of the PPR in each State/UT of India and to be screened by using an approved PPR C-ELISA kit.

Sampling plan for Sero-monitoring of Peste des Petits Ruminants

Peste des petits ruminants (PPR), otherwise known as 'Small ruminant plague' or 'Goat plague', is an acute, highly contagious, World Organisation for Animal Health (WOAH/OIE) notifiable and transboundary viral disease of sheep and goats. PPR is caused by the small ruminant morbillivirus (SRMV), formerly known as PPR virus (PPRV). Clinically, the disease is characterized by high fever (pyrexia), discharges from eyes and nasal orifices, necrotizing and erosive stomatitis, gastroenteritis, diarrhoea, and bronchopneumonia. The disease poses a substantial threat to the small ruminants' sector and it significantly impacts the food security and national economy of the country. In enzootic India, the disease causes major constraints in augmenting the productivity of sheep and goats with an annual estimated financial loss of Indian rupees (INR) ~16118 million. After the eradication of rinderpest, a global consensus on PPR Global Control and Eradication Strategy (GCES) has been reached on the need to control and eradicate the disease by 2030 due to its economic significance. The recent success with the rinderpest eradication programme (NPRE) in the country has provided the confidence and impetus

to launch a similar programme for PPR. India initiated the national control programme for PPR (PPR-CP) in 2010-11 using its resources based on the available epidemiological data, indigenously developed robust diagnostics, and vaccines to mitigate the disease burden even before the global framework was developed. PPR control and eradication depends mainly on rapid and accurate diagnosis or surveillance/monitoring and implementation of the prompt vaccination programme.

Department of Animal Husbandry and Dairying, the Government of India has taken the initiative to control PPR by vaccination and evaluating its efficacy by sero- monitoring and surveillance in the National PPR Control and Eradication Strategic Plan 2030-(PPR-EP) Considering the huge livestock resources in India, while studying vaccination efficacy, sampling plan is very important for systematic collection of representative samples and thereafter testing in laboratories for seromonitoring & surveillance of PPRV antibodies in the small ruminants population. The sampling plan provided in this handbook follows OIE guidelines for the collection of samples from different parts of the country to be tested for PPR vaccine or PPRV infection-induced antibodies response under PPR-EP and to be used in the CADCP programme in India.

A cross-sectional seroprevalence study is to be conducted to ascertain the prevalence status of PPR virus antibodies in small ruminants population in the epidemiological units (epi- units) of different states in India, for surveillance or seromonioring of PPR in India. The initial hypothesis is that PPR antibodies are homogeneous or independent in the populations of the epi-units in the study region. The rural societies live in villages consisting of clusters of households that follow similar animal husbandry and socio-economic activities. Hence, the village is considered a distinct epi-unit in this study as described earlier. Accordingly, a list of villages in various blocks/ tehsils in different districts in the state and their sheep and goat populations as per the 20th Livestock census, 2019 in each of the states was prepared and to have a sizeable population, the villages (epi-units) having more than 100 or 200 or 500 sheep and goats (with inclusion and exclusion criteria, as the case may for each state) were shortlisted for the sampling frame.

The sample size was determined as per Cochran, (Cochran 1963) formula by using epitool. For the calculations of the Sample cluster unit (epi-unit), the indigenous PPR Competitive ELISA diagnostic sensitivity (92.4%) and diagnostic specificity (98.4 %), were considered, along with a 95% Confidence Interval (CI) and 5% or 10% standard error. To calculate the sample size of epi-units with a specified level of confidence (CI) and precision, assuming an unknown large population of epi-units, the formula n = (Z^2 × P (1 – P))/e2; Z = Value from the normal distribution; P = Expected proportion of epi-units protected; e= Desired precision is used. where n is the sample size for the state, Z is the 95% confidence level (standard normal value of 1.96), p is the prevalence, which is to be taken as 30 % for pre- vaccination sero-monitoring and 70% will be taken as immune population/ herd immunity at field level for post-vaccination sero-monitoring. ‘e’ is the precision of sample size estimate and is normally set at 5% (0.05) or

10 % (0.10), as is the acceptable sampling error, as per OIE/FAO-Global Control and Eradication for PPR (GCEP) guidelines (OIE and FAO 2015). Based on these input parameters, the total number of sample sizes (Epi-units) within a study area were estimated for PPR sero-monitoring /surveillance in a particular state. Further, to account for variation in the sensitivity/specificity of diagnostic test/assay, the formula has been modified, and accordingly, the sample size estimation for identifying the number of clusters or villages or epi-units to be carried out was done by epi-calculator. Using the above formulae with the following inputs, viz., the Unit level design prevalence of 30 %; cluster-level design prevalence (3%), and target cluster sensitivity (92.4 %), the sensitivity of the test /assay to be employed (92.4 %) and with target system sensitivity of 95 % confidence level, **the estimated sample size -** Epi-unit clusters to be sampled for pre-vaccination / post-vaccination / population immunity assessments was minimum of **108 (Villages or Epi-units)/study areas (state or UT) with a maximum of 120 villages/state or UT.**

For PPR sero-monitoring /surveillance, a multistage random sampling technique is to be employed to collect serum samples from sheep and goat-rearing households/ flocks as per WOAH(OIE)/FAO guidelines. The Multi-stage random sampling technique with a fixed level or the required number of clusters units / epi-units regarding a small ruminant population where PVE is implemented to be carried out with random allocation.

Considering the huge small ruminants (~20 million) resources in India, in the first stage, the study area was purposefully selected (as the state), where PVE needs to be evaluated. In the second stage, the districts where the vaccination programme is implemented in a particular state need to be selected. In the third stage, in each of the districts, villages as an epidemiological unit (epi-unit) to be selected randomly. Randomization of villages was done based on the in-house NIVEDI-developed software epi-calculator. Before randomization, the trimming of the sampling frame (source of villages from which samples are to be drawn) is employed for 500 animals per village as an inclusion criterion. However, in some of the villages with less sheep and goats population, the inclusion criteria will be 100 or 200, or 300 animals per village, as the case may be for each state. The required number of epi-units needs to be calculated and allocated randomly among the districts.

In the fourth stage, the flocks or households are to be surveyed in each of the selected villages or epi-unit. The selection of flock/household within the village will be done based on systematic random sampling with prior preparation of sampling frame (flocks) having a minimum of 20 animals. In the next stage in each of the selected villages, the number of secondary units (animals) was calculated by the hypergeometric distribution as per GCES guidelines by considering an animal unit-level prevalence of 30% (OIE FAO 2015b) in small ruminants. In each epi-unit/village, sera are to be collected randomly from 3 or 4 flocks or households. The maximum level of 9 to 10 samples to be collected was determined based on the small ruminants populations in each epi-unit. In the epi-unit, where only either sheep or goats were present, a maximum of 9-10 samples of either species were to be collected. Maximum of 3 animals

per each eligible age group or stratum (age = between 6-12 months, one to 2 years and > 2 years) or 3 animals in 6-12 months age group and 6 animals in more than one-year age group, in each flock/household, with a total of 9 or 10 samples from each selected flock to be collected. Thus, a maximum of **3240 secondary animal units (108 Villages x 30 samples in each village) to be collected for** surveillance of the PPR in each State/UT of India was established using the epi-calculator https://www.nivedi.res.in/Nadres_v2/Epical/stratified/random_sampling.php.) **for pre- vaccination or population immunity** assessments.

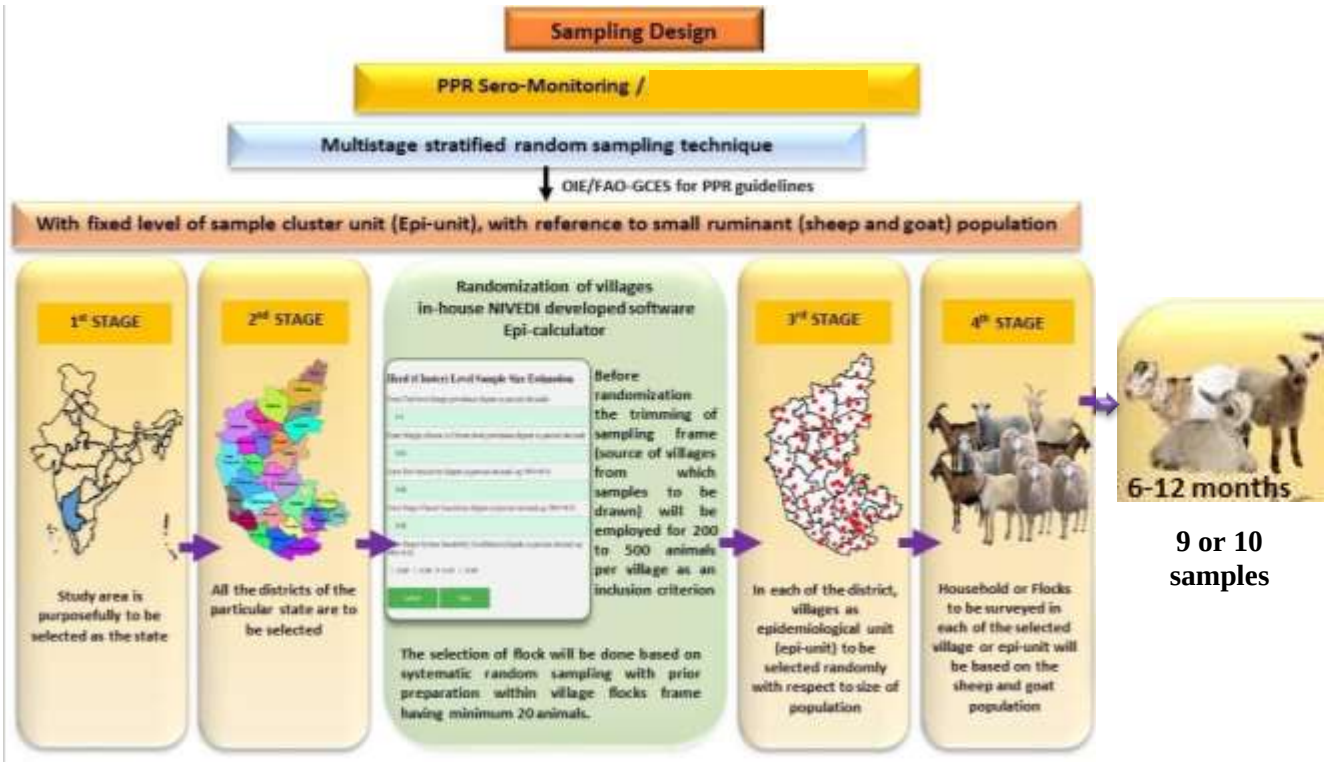
For post-vaccination assessment, a **total of maximum 9 to 10 samples from each of the selected villages / epi-unit to be collected randomly from 6 to 12 months aged animals only,** from three or four selected flocks, with earlier described population and species proportion ratios in the **provided sampling plan for each State/UT as such directly**. Thus, a maximum of **1080 secondary animal units (108 Villages x 10 samples in each village) are to be collected for** seromonitoring of the PPR in each State/UT of India.

The collected sera to be labeled and transported in an ice-cool shipment container to the designed laboratory and upon receive samples to be stored at -20 °C until further testing for PPRV antibodies. The seromonitoring and surveillance of the PPR in India will be carried out by using an approved PPR C-ELISA kit, by the DAHD, GoI.

For example for Karnataka State- Seromonitoring Multistage random sampling methodology employed

Survey Area	**Country**	**India**	**Remarks**
Ist stage	State	Karnataka	The study area is purposefully selected as the state
2nd Stage	Districts	All districts	All the vaccinated districts of the particular state selected
3rd Stage	Villages /Epiunits	Minimu 108 Maximum 120 villages randomly to be selected	Before randomization, the trimming of the sampling frame (source of villages from which samples are to be drawn) will be employed for 500 or 200 to 100 animals per village as an inclusion criterion. In villages as an epidemiological unit (epi-unit) to be selected randomly concerning the size of the population

4th Stage	Household or Flocks	3 or 4 flocks in the villages	Based on the small ruminants population, a maximum of 9 or 10 animals per eligible age group (age= between 6-12 months)
Total no. of samples	**Minimum samples size = 108 x 9 = 972 samples or Maximum samples size = 108 x10= 1080 samples per State**		



Sampling Plan Details for Surveillance and monitoring and of PPR under PPR-EP, CADCP , LH&DCP Scheme, DAHD, GoI.

Surveillance and Seromonitoring in natural hosts – Sheep and Goats

1st Year

I. Baseline Serosurvey (pre-vaccination): Serosurvey (PPR Sampling plan 1) will be carried out in different age groups (i) 6-12 months; ii) 1 year to 2 years iii) > 2 years to know the status of the PPRV antibodies in sheep and goat population (base-line seroprevalence of PPR) due to the impact of the ongoing PPR-CP implemented in various states of India, as different states are at a different level of implementation of the PPR-CP.

II. Seromonitoring (post-vaccination): Serosurvey will be done targeting only for the animals of 6–12 month age group **(PPR Sampling plan 2)** within 90 days after two months of vaccination drive (between 60- 90 days after vaccination) undertaken to know the efficacy of the vaccination at field scenario.

III. Serosurvey will be taken in sheep & goats (PPR Sampling Plan 3) after 8 months of vaccination to assess the status of PPRV antibodies in the age group of 1 year and above (part (i) 1 year to 2 years part ii) > 2 years age groups).

2nd year

I. Seromonitoring will be done for the age group of 6-12 months (PPR Sampling Plan 4) after vaccination within 90 days after two months of vaccination drive (between 60- 90 days after vaccination) to know the efficacy of the vaccination

II. Serosurvey for Population immunity in sheep & goats (PPR Sampling Plan 5) will be assessed after 8 months of vaccination in the age group of 1 year and above (part (i) 1 year to 2 years part ii) > 2 years age groups).

3rd year- same as above -details mentioned in 2nd year (sampling plan 6 and 7)

4th year- same as above -details mentioned in 2nd year (sampling plan 8 and 9). A review of population immunity/herd immunity at the end of 4 years will be assessed and analyzed with progressing outbreaks and syndromic surveillance information to decide about the further course of action required for successful PPR-EP.

Terminologies used in the seromonitoring and surveillance of PPR

Sampling Plan

The term "sample" may either mean a specimen (animal, blood sample, and others) or, used in the statistical sense, a sub-collection or subset of units. The aim, in general, is the collection of random, representative, and independent samples from the study population, as it is difficult to collect materials required for disease monitoring /surveillance from all the animals in a population. Sampling plans/designs vary depending on the problems to be addressed to the study. The general principles of PPR serological surveys are to evaluate vaccination effectiveness or Post Vaccination Evaluation (PVE) tool attempts to answer the following points/hypothesis.

- ✓ To establish the baseline level of epi-units that have been exposed to the PPRV before vaccination in the target population (***Prevaccination assessment***).
- ✓ To evaluate vaccination effectiveness by estimating the number of epi-units that demonstrate appropriate seroconversion after each round of vaccination (***Post-vaccination effectiveness***).
- ✓ To evaluate population immunity (number of epi-unit protected) at a given time and over time after several vaccination campaigns by comparison with the results of the baseline survey (***Population Immunity assessment***).
- ✓ To determine age strata (group), which have been protected for PPR.

Target Population:

It is defined as all susceptible small ruminants at risk of PPR in a particular location.

Study population:

It is defined as epidemiological units (epi-units)/ villages included in the vaccination programme. Animals in the epi-units can be stratified by different age groups /strata. Below 6 months; 6-12 months, 12-24 months, and > 2 years.

Below the 6-month group is excluded from the post-vaccination evaluation, like 0-3 months age group during the post-vaccination survey will remain as unvaccinated animals, as recommended age of vaccination is either at 3 months or 4 months old.

Using three age groups more precise information will be assessed on the particular strengths or weaknesses of the vaccination campaigns, as certain age groups might have been favoured by farmers when presenting the animals for vaccination. If, however, the primary objective is the evaluation of the overall protection of epi units without considering differences between all age groups, two groups will suffice: i.e., one group less than one year (6-12months) and another group >1 year.

Epidemiological unit:

The epi-unit is defined considering that all small ruminants within each unit will have the same chance of being vaccinated and the same risk of being infected (or have specific antibodies against PPR).

Generally, in the Indian context, the village is as considered the epi- unit, as the rural societies live in villages consisting of clusters of households that follow similar animal husbandry and socio-economic activities.

- Please refer to the guideline **Annexure -1** while collecting the blood samples from the animals, the animal data (Model table sheet provided), and the collection of samples from epi-units for seromonitorting and sero-surveillance
- Please refer to the guideline for the collection of samples from epi-units **(Annexure 2)**
- Please refer to the guidelines for collection, storage, and transportation of samples **(Annexure 3)**

As the country is going to adopt seromonitoring and surveillance of PPR at the national level in consonance with the GOI plan of PPR national control and eradication strategy 2030, it is recommended that the sampling plan provided in this handbook be endorsed by the OIE official before implementation as it is planned for the eradication of PPR from India.

Reference

OIE, FAO. Manual on global strategy for the control and eradication of Pesti des petits ruminants and Annexe 3.4. Post Vaccination Evaluation Tool, 2015. https://www.fao.org/3/a- i4460e.pdf.

Annexure-3

भाकृअनुप–राष्ट्रीय पशुरोग जानपदिक एवं सूचना विज्ञान संस्थान
ICAR–National Institute of Veterinary Epidemiology and Disease Informatics
रामगोंडनहल्ली, येलहंका, बेंगलुरू – 560064
Ramagondanahalli, Post Box No: 6450, Yelahanka, Bengaluru - 560064

Action Plan for PPR surveillance and monitoring under (PPR-EP) CADCP, (LH&DC Scheme), DAHD, GoI

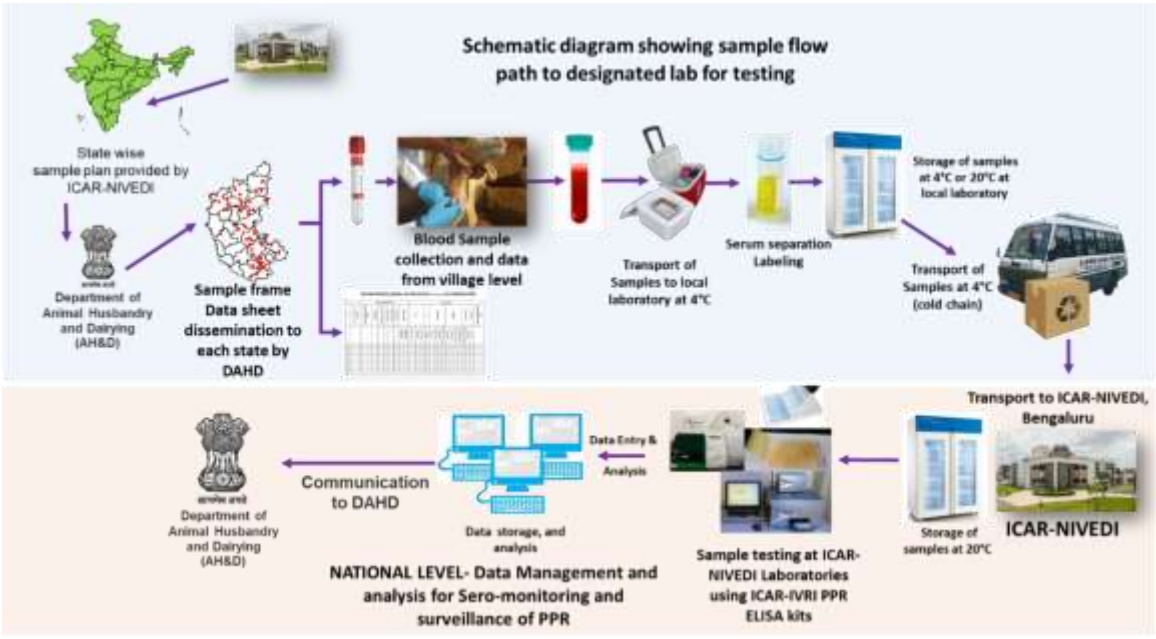
Sero-monitoring of Peste des Petits Ruminants

Collection and Storage of samples

- Sample collection is done for definitive serological diagnosis/monitoring/surveillance of PPR.
- Before the collection of blood, make the area of skin surface sterile by using alcohol swabs. Collect blood from animals using a sterile vacutainer.
- Collect 5 to 8 ml of blood per animal which can be used for various tests and diagnoses of disease.

- Collected blood samples in vacutainer tubes for seromonitoring and surveillance (Heparin/EDTA), to be labeled and label details recorded in each animal's details with the unique ID of the samples (**Annexure 1 to be referred**). The tubes need to be placed in a cool shipment box with ice packs and transported to the laboratory and upon receipt, samples need to be stored at 4°C until further use.
- For serum, collect the blood in a tube coated with sodium silicate vacutainer tubes which favors clot formation and easy separation of serum. The serum should be separated by centrifugation at 2000 rpm for 15 to 20 minutes.
- The serum collected may be used for serological investigations and it should be stored in two aliquots at -20°C and can be used for the detection of PPRV antibodies for seromonitoring and surveillance as well as for population immunity assessments.
- The collected samples' details are to be entered in the data sheet format (**Annexure 1 to be referred**) for subsequent retrieval of data, sharing, and analysis for seromonitoring / surveillance

and evaluation of the PPR-EP programme.



Transport of samples

- Do not transport material for serological investigations in the needle and syringe. Needle transport is very unsafe because there is always the risk of a needle stick injury, and syringe transport poses a risk because specimens may be expelled during transport, creating a threat to personnel and the environment. Transfer aspirated material to a tight container.
- The sample containers should be properly labeled and identified. The labels like “Clinical Specimen/Blood/Serum samples, Fragile and Handle with care” may be specified on the container or the parcel containing the samples.
- Blood samples for disease diagnosis should be collected during outbreak investigation in Heparin/EDTA vacutainer tubes and should be transferred in the inner thread screw cap 1.8 or 2 mL vials properly without leakage for transport.
- The serum samples after separation should be stored **in the inner thread screw cap** 1.8 or 2 mL serum cryovials properly without leakage for transport.
- The serum sample container should have details like the nature of the sample, epi-units ID, animal number, place, and date of the collection which is important for easy identification and tracking of samples (**Annexure 1 to be referred**).
- The samples should be sent with proper sample details in a separate sheet which will help in the testing of the samples for PPR by ELISA or other tests.
- The serum samples should be transported on the ice at 4°C to prevent the decomposition of the serum proteins. The samples are to be stored upon arrival if the processing/transport is delayed

and are usually done using refrigeration.

- The serum can be stored at freezing conditions at 0°C or -20° C for a long time storage without decomposition of the serum proteins.

Instructions/Note:

- The proper and systematic collection, dispatch, and storage of samples are necessary and given at most care for proper diagnosis/ monitoring /surveillance of animal diseases.
- The efforts made in diagnosis/monitoring will go waste if proper or representative samples are not collected. Hence, the collection of samples should be given more importance for proper diagnosis/ monitoring of animal diseases.
- While collecting the blood samples from the animals, the animal data (Model table sheet provided- **Annexure -1 (Village/Epi-Unit- Samples collection Sheet)** compulsory should be entered.
- Labeling of serum sample vials should be written as Starting letter of village name followed by 1 to 10 for the given in the sample frame for the sampling plan for the given year with the purpose. For Example- village name Hosakote, serial no 1: then the sample ID will be "H1"
- All the serum samples after separation should be stored **in the inner thread screw cap** 1.8 or 2 mL serum cryovials properly without leakage and can be stored at -20 ℃ in the deep freezer.
- All the labeled serum samples vials (10 Samples) of a particular village should be packed in a single plastic Ziplock pouch and labelled/sticker should be placed over the pouch (Labeled with State, District, Block, and **Village name**, as per sampling frame) and to be transported to the designated laboratory in the cold chain at 4 °C(Ice Pack).
- Please refer to the guideline for the collection of samples from epi-units **(Annexure 2)**
- Please refer to the guidelines for collection, storage, and transportation of samples **(Annexure 3)**