



# Sampling Plan for Serosurveillance of FMD in Small Ruminants in Karnataka under National Animal Disease Control Programme (NADCP) - 2024



## **ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI)**

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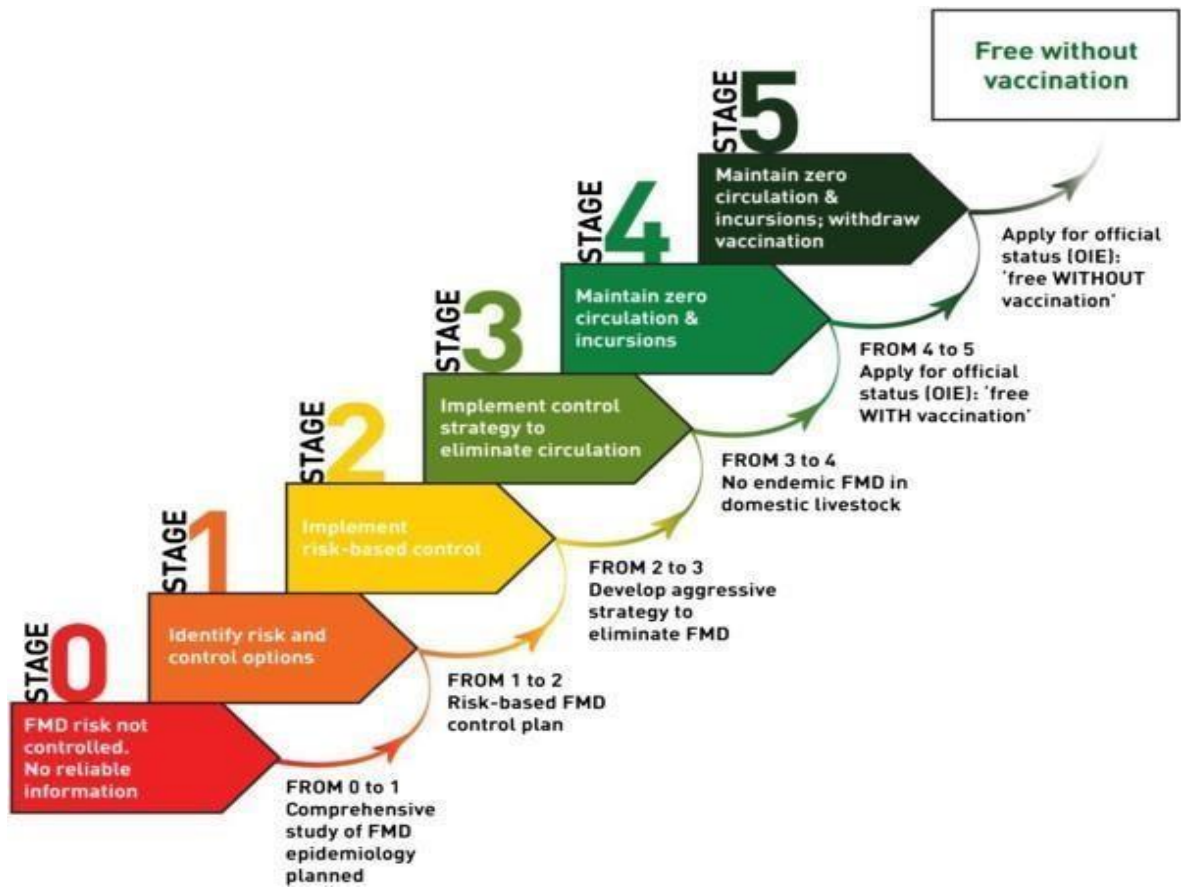
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## Table of Contents

I. Introduction.....	1
1. Sampling Plan for Serosurveillance of FMD in Small Ruminants in Karnataka.....	11
2. Reference.....	13

## **Introduction:**

Livestock plays a pivotal role in the Indian economy, as it is an essential sector of agriculture providing income to the farmers directly or indirectly. Sustainable growth of the livestock sector can be achieved by increasing nutritional security and controlling major livestock diseases. Foot-and-mouth disease (FMD) is a clinically acute, contagious disease of cloven-footed animals (cattle buffalo, sheep, goat, pig) and causes a severe threat to the livestock economy. Karnataka state has a considerable FMD susceptible population of 29 million animals which includes Cattle (8.45 million), Buffalo (3.0 million), Goat (6.2 million), Sheep (11 million), Pig (0.3 million). FMD causes significant economic losses due to the mortality of young animals, drop in the milk production, declined productivity, and international trade restrictions. In India, the annual direct loss due to FMD is 20897 crores. As FMD is endemic in India, control strategies have the foremost importance for protecting the livestock population and revamping farmers' livelihood.

FMD control demands strict zoo sanitary measures and vaccination. Low vaccination coverage, multiple serotypes, poor cold chain maintenance infrastructure in rural areas, and unrestricted movement of animals exacerbate the control program in India. Continuous surveillance, disease monitoring, vaccine matching, and vaccine quality improvement will significantly change the Indian scenario of FMD control. In 2003-2004, the Government of India launched FMD control program with regular vaccination to prevent economic losses due to the disease and develop herd immunity in cattle. The FMD control programme is being implemented in the state since 2011, where all the eligible cattle and buffalos are vaccinated twice a year. At present the FMD vaccination programme is carried out under National Animal Disease Control Program of Government of India.

The systematic vaccination of cattle and buffaloes against FMD has resulted in considerable reduction in the number of clinical diseases, as well as number of animals getting affected. The Seromonitoring of vaccination has revealed that there is gradual building up of herd immunity in the population. The serosurveillance under National FMD Serosurveillance program has revealed reduction in infection status among cattle and buffaloes.

The small ruminants are equally susceptible to FMD, but these are not vaccinated under FMD control programme. In recent times, outbreak of FMD has been confirmed and is increasing in small ruminants. Hence, there is a necessity to study the infection status among small ruminants too. This will help to implement the strategy of FMD control in small ruminants. Therefore, random serum samples are collected from small ruminants across the state and screened by DIVA-ELISA to know the infection status and virus activity in these population.

## **Sampling Strategy currently used for Sero-surveillance**

All form of epidemiological investigations requires the scientific sampling plan for collection of data on health problems. Estimation of prevalence of a disease is a prerequisite to establish the disease control program, hence sampling the populations in order to estimate the level of disease prevalence is common task for epidemiologists. In sampling, one should ensure that animals are representative of the target population so that the estimated value/s are unbiased and precise (low standard error). Simple random sampling, systematic or stratified random samplings are the most commonly used sampling methods.

In the developed world, which practice two stage sampling, the individual livestock farm forms the primary sampling unit. However, this concept cannot be adopted in our country as farm wise details are not available to prepare a sampling frame. Additionally, in a country where small farms (2-3 animals/household) are a norm, the farm becomes too small a unit to do the sampling. In such a situation, taking the village as primary sampling unit makes sense as fairly homogeneous population of animal exist in a village and these form a natural cluster. It is argued that in a large population where animals are separated into herds (villages in our context), disease has a strong tendency to cluster. This is because the disease agent or agents (whether infectious, environmental or genetic) are generally not evenly distributed throughout the population. With rare diseases, this clustering is usually even more pronounced. As a result, a particular disease may affect a very low proportion of herds-but within those affected herds, the prevalence of the disease amongst animals may be quite high. If a survey designed to detect the presence of disease fails to take into account the clustering of disease in the population, the results of the survey are likely to be very unreliable. This is because the probability formulae that the surveys are based on assumption that every unit in the population has the same probability of being affected. Another problem with large-area surveys is the logistics of sampling. The solution to both these problems is to use a two- stage sampling strategy in which villages form the primary sampling units (first stage), and individual animals within selected villages make secondary sampling units (second stage). In this way, the sample sizes at each stage can be adjusted to reflect the different disease prevalence's (the proportion of villages affected in the first stage, and the proportion of animals affected in the village at the second stage). Two-stage sampling also means that the construction of sampling frames is much simpler. At the first stage, only a list of all villages (defined epi unit) in the population is required, and at the second stage, only animals in each of the selected villages are to be included in the list.

In this sampling scheme we employed two stage stratified random sampling, with district as stratification variable for Sheep & Goat population in different states. The present sampling strategy is a deviation from the previous sampling methodology. The input requirements used for sampling plan were;



- a. The estimated animal level prevalence for NSP protein ( Data obtained from surveillance of 2023) as shown in Table 1,
- b. The requirement of Cluster or Village level proportion was derived by adjusting the district level population and levels of herd immunity of previous data of Seromonitoring so that herd level prevalence is proportional to the probability of population size.
- c. The sampling plan strategy is accounted for variations in diagnostic sensitivity and specificity for NSP protein.
- d. Village level livestock population has been set with minimum criteria of population size for deriving the village random sample, so as to enable to have good compliance rate of sampling.
- e. Village level livestock population frame (list of villages with livestock population) was created without replacement (WOR) strategy, which takes into account of villages already sampled during the previous surveillance plans. State level frame was stored in csv files with pre-defined state and district level codes for generating plans.
- f. The standard imputation technique was implemented for estimating the missing animal and cluster level prevalence for the states for which data is unavailable.

Two -stage stratified random sampling plan was generated at reasonably high confidence (0.9) using in-house developed using epi-calculator under NADRES v2 by ICAR-NIVEDI, Bengaluru. ([https://www.nivedi.res.in/Nadres\\_v2/Epical/stratified/random\\_sampling.php](https://www.nivedi.res.in/Nadres_v2/Epical/stratified/random_sampling.php)). The summary of sampling plan generated using two-stage stratified random sampling scheme is presented in Table 2 &3. The Phase II of Sampling plan was done for the year 2023 and the results obtained from the DIVA-ELISA are presented in the Table 1.

### **Choosing random samples in selected village**

It is to suggest that the random approach must be adopted to choose the animals with in the selected village. It is better to divide the selected village into four to eight directional part (viz. North, East, South, West, North-East, South-East, South-West and North-West) and each directional part equal number of animals are required to be chosen randomly. The number of animals to be chosen for each selected village is mentioned in the respective state-wise sampling plan.

For example, 16 number of animals are to be drawn from the selected villages, 2 animals to be drawn randomly from each direction of village.

Table 1: Results obtained from DIVA –ELISA test for SeroSurveillance of FMD in Small Ruminants in Karnataka 2023.

<i>Sl no</i>	<i>Name of District</i>	<i>Total no of serum samples tested</i>	<i>No. of Samples positive for DIVA test</i>	<i>Percentage prevalence (CI)</i>
1	Bagalkot	18	0	0
2	Bangalore Rural	27	0	0
3	Bangalore Urban	27	4	14.8
4	Belgavi	72	5	6.9
5	Bellary	18	1	5.5
6	Bidar	9	1	11.1
7	Chikkaballapur	54	0	0
8	Chikmagalur	27	3	11.1
9	chitradurga	27	0	0
10	Dakshina Kannada	18	0	0
11	Davangere	9	0	0
12	Dharwad	18	2	11.1
13	Gadag	17	0	0
14	Hassan	63	0	0
15	Haveri	27	4	14.8
16	Kalaburagi	45	0	0
17	Kodagu A	9	1	11.1
18	Kodagu B	9	0	0
19	Kolar	54	2	3.7
20	Koppal	10	3	30
21	Mandya	45	0	0
22	Mysuru	45	0	0
23	Ramanagara	18	2	11.1
24	Shivamogga	63	13	20.6
25	Tumkuru	72	3	4.2
26	Uttara kannada	63	2	3.1
27	Vijayanagara	9	0	0
28	Vijaypur	18	2	11.1
29	yadgiri	18	3	16.6
	<b>Total</b>	<b>909</b>	<b>51</b>	<b>5.6</b>

Table 2: Summary of sampling plan using Two-stage stratified random sampling for SeroSurveillance of FMD in Small Ruminants in Karnataka 2024.

STATE	NO OF DISTRICTS	NO OF BLOCKS	Total no of Sheep + Goat **	No of Animals to be Sampled	Average No of Samples Per Districts
Karnataka	29	93	17220120	1588	55

**\*\* 20<sup>th</sup> Livestock Census (DAHD, GoI)** *Animals within 6-18 months of age must be sampled.*

**Note:** *Samples to be collected preferably within 1-2 months before vaccination or 4-5 months after vaccination*

Table 3: Summary of district wise sampling plan using Two-stage stratified random sampling for SeroSurveillance of FMD in Small Ruminants in Karnataka State 2024.

<i>Sl No</i>	<i>District Names</i>	<i>No of Blocks (to be sampled)</i>	<i>Total no of population (Sheep + Goat) **</i>	<i>Total No of Sheep and Goat population in the sampled villages</i>	<i>Total no of samples to be Drawn</i>
1	Bagalkot	1	1006782	2032	17
2	Bangalore Rural	3	213944	1176	51
3	Belgaum	6	1459420	13218	103
4	Bellary	2	1258684	8134	35
5	Bengaluru Urban	2	145337	685	60
6	Bidar	2	268802	1220	34
7	Bijapur	2	916168	2676	34
8	Chamarajanagar	2	279954	1346	34
9	Chikballapur	5	801585	3595	85
10	Chikmagalur	2	139002	1067	34
11	Chitradurga	3	1737145	29617	53
12	Dakshin Kannad	2	32504	542	34
13	Davangere	2	630172	2462	34
14	Dharwad	2	153938	893	34
15	Gadag	2	587555	3169	34
16	Gulbarga	3	558587	2420	51
17	Hassan	7	328445	6919	120
18	Haveri	2	458174	2490	34
19	Kodagu	2	8253	322	34
20	Kolar	5	577605	2352	85
21	Koppal	2	797945	2918	34
22	Mandya	5	693563	2880	85
23	Mysore	5	411669	2452	85
24	Raichur	2	940351	2461	34
25	Ramanagara	2	278118	1155	34
26	Shimoga	5	102245	1343	85
27	Tumkur	8	1717934	7887	137
28	Uttar Kannada	5	19192	1029	85
29	Yadgir	2	693940	4113	35
	<b>Total</b>	<b>93</b>	<b>17217013</b>	<b>112573</b>	<b>1588</b>

## **Standard Operating Procedure (SOP) for Collection and Dispatch of Sheep and Goat Serum under National FMD Serosurveillance**

**General requirements:** Aseptic precautions should be observed right from collection of blood to dispatch of serum. It is advisable to wear gloves at all times (be it removal of stopper from vacutainer, centrifugation, pipetting, disposal of contaminated tubes, and clean-up of any spills) while handling the specimens. Used vacutainers, needles, and pipets must be properly disposed in accordance with biosafety/institutional requirements.

### **Serum Collection**

#### Sample collection

Samples were collected from sheep and goat

#### Materials required

- Vacutainers 5 ml capacity (Red Top, BD Catalogue No. 366430)
- Desktop Centrifuge (refrigerated preferably) with swinging bucket rotor
- Sterile 15ml polypropylene centrifuge tubes
- Sterile Cryovials (preferably internal threaded)
- 2ml, 5ml pipettes or 1ml micro pipettes and 1 ml sterile pipette tips.
- Ice Buckets
- Laminar Flow cabinet

#### Serum Separation Procedure

- ❖ Keep the vacutainer in a slanting position after the blood is drawn (draw blood only up to 2/3 of the maximum volume) at room temperature for a minimum of 30 to a maximum of 60 minutes to allow the clot to form.
- ❖ If the blood is not centrifuged immediately after the clotting time, the tubes should be placed on ice or refrigerated. (4°C).
- ❖ Transfer the content of the vacutainer to a sterile 15 ml centrifuge tube (if you are doing this in the lab kindly use laminar flow).
- ❖ Centrifuge the blood sample at in a swing-out rotor for 20 minutes at 1100-1300 g at room temperature.

**Warning:** Excessive centrifuge speed (over 2000 g) may cause tube breakage

- ❖ At the end of the centrifugation transfer the serum (Recommendation: do not pour!) to a sterile labelled cryovial (preferably internal threaded) using a pipette. Close the caps on the vials tightly.

**Note:** It would be wise to keep the pipet above the red blood cell layer and leave a small amount of serum in the tube so as avoid picking up red blood cells.

- ❖ Check that all aliquot vial caps are secure and labelled.
- ❖ Place all aliquots upright in a specimen box or rack in  $-20^{\circ}\text{C}$  or colder freezer. All specimens should remain at  $-20^{\circ}\text{C}$  or below prior to shipping.

### Serum Shipping Instructions

- ❖ Use at least 1-inch thick walled thermocol box for shipping of serum
- ❖ The serum samples should be preferably shipped in dry ice, if not add sufficient number of ice packs which were earlier stored in  $-80^{\circ}\text{C}$ . If ice packs are used for shipping, ensure that samples are placed within layer of icepacks.
- ❖ Kindly ensure that there is no leakage during shipment. To do this it wise to keep the vials in a cryo box.
- ❖ Ship the serum as quickly as possible, preferably by overnight courier.
- ❖ Inform DFMD/relevant laboratory through telephone call about the shipping.
- ❖ Ensure that the samples are accompanied by standard proforma attached herewith in appendix II (proforma for serum dispatch to ICAR-DFMD/Relevant Lab)

### **Please contact for any clarifications on SOP and Sampling plan issues:**

1. Dr. K.P. Suresh, Principal Scientist : 7892791174
2. Dr. Raveendra Hegde, Director : 9448358705

**Proforma for serum collection in the field**

**Sample ID:**

**Date of Sampling (DD/MM/YYYY):**

**Owner's Name:**

**Mob No.:**

**Village:**

**Tehsil/Block:**

**District:**

**State:**

**Pin code:**

**Species of animals (Tick the correct one)**

Sheep

Goat

**Sex (Tick the correct one)**

Male

Female

**Age in Months (write the age)**

**Health status of the animal (Tick the correct one)**

Apparently Healthy

Diseased: Yes/No

Suspected disease (If Yes):

**Vaccination History**

Vaccinated/not vaccinated

**Date of vaccination**

**Remarks if any:**

**Proforma for Serum Dispatch to FMD Regional Centre, IAH&VB,  
Bangalore**

**Ref No.:**

**Date:**

**Sender:**

<b>Sl. No.</b>	<b>Sample ID</b>	<b>Date of Sampling</b>	<b>Owner's Name</b>	<b>Mob No.</b>	<b>Village</b>	<b>Block/Tehsil</b>	<b>District</b>	<b>PIN Code</b>	<b>Animal Species</b>	<b>Age in Months</b>	<b>Sex</b>



## SRK/SEROSUR/NIVEDI/Phase-III/Karnataka\_16/2024

## Stratified Random Sampling:

Species Selected for Stratification = Sheep + Goat

Number of Villages Having 100 + (Sheep + Goat) = 19261

Animal Level Prevalence = 0.15

Cluster Level Prevalence = 0.03

Sensitivity of the test used = 0.9

Total No of Villages (Clusters) Selected = 93

Total No of Animals to be Sampled = 1588

DISTRICT_NAME	BLOCK_NAME	VILLAGE_NAME	Goats	Sheep	Sheep + Goat	Number of units to sample	Goat Proportion	Sheep Proportion
Bagalkot	Badami	Ugalawat	873	1159	2032	17	7	10
Bangalore Rural	Hosakote	Ittasandra	113	215	328	17	6	11
Bangalore Rural	Dod Ballapur	Mukkadigatta	108	274	382	17	5	12
Bangalore Rural	Hosakote	Oblahalli	161	305	466	17	6	11
Belgaum	Hukeri	Hukeri (Rural)	74	56	130	17	10	7
Belgaum	ARABHAVI	Arabhavi-Ward No.4	264	0	264	17	17	0
Belgaum	Belgaum	Bhendigeri	270	814	1084	17	4	13
Belgaum	Gokak	Avaradi	636	565	1201	17	9	8
Belgaum	Hukeri	Wantmuri	1385	488	1873	17	13	4
Belgaum	Raybag	Nidagundi	3646	5020	8666	18	8	10
Bellary	Hospet	Danayakanakere	438	1391	1829	17	4	13
Bellary	Bellary	Yalpi	1426	4879	6305	18	4	14
Bengaluru Urban	Bangalore North	Challahalli	52	200	252	17	4	13
Bengaluru Urban	Bangalore North	Shivakote	135	298	433	17	5	12
Bidar	Aurad	Torna	441	41	482	17	16	1
Bidar	Bidar	Ghodepalli	585	153	738	17	13	4
Bijapur	Indi	Miragi	967	0	967	17	17	0
Bijapur	Indi	Bhuyar	958	751	1709	17	10	7
Chamarajanagar	Chamarajanagar	Bandigere	189	201	390	17	8	9
Chamarajanagar	Kollegal	GANIGAMANGALA	676	280	956	17	12	5
Chikballapur	Chikballapura	Gudisihalli	186	167	353	17	9	8
Chikballapur	Chintamani	Bhoomi Setti Halli	64	471	535	17	2	15
Chikballapur	Bagepalli	Kothakote	179	417	596	17	5	12
Chikballapur	Gauribidanur	Pothenahalli	314	679	993	17	5	12
Chikballapur	Gauribidanur	H.Nagasandra	310	808	1118	17	5	12
Chikmagalur	Tarikere	Udeva	209	147	356	17	10	7
Chikmagalur	Kadur	G.Thimmapura	105	606	711	17	3	14
Chitradurga	Hosdurga	Ajjikamasagara	125	885	1010	17	2	15
Chitradurga	Hosdurga	Doddabyladakere	367	2130	2497	18	3	15
Chitradurga	Hiriyur	Pilali	3387	22723	26110	18	2	16
Dakshin Kannad	Bantval	Netlamudnur	236	0	236	17	17	0
Dakshin Kannad	Bantval	Phajeer	306	0	306	17	17	0
Davangere	Channagiri	Maravanji	341	642	983	17	6	11
Davangere	Jagalur	Doddabommanahalli	152	1327	1479	17	2	15
Dharwad	Hubballi-Dharwad	Hubli-Dharwad (M Corp.) - Ward No.29	193	109	302	17	11	6
Dharwad	Hubli	Umachigi	395	196	591	17	11	6
Gadag	Ron	Kuntoji	49	1202	1251	17	1	16
Gadag	Shirhatti	Bellatti	779	1139	1918	17	7	10
Gulbarga	Aland	Munhalli	454	20	474	17	16	1
Gulbarga	Afzalpur	Atnoor	872	43	915	17	16	1
Gulbarga	Chincholi	Hodebeerahalli	479	552	1031	17	8	9
Hassan	Hole Narsipur	Ganjikuppahalli	18	101	119	17	3	14

DISTRICT_NAME	BLOCK_NAME	VILLAGE_NAME	Goats	Sheep	Sheep + Goat	Number of units to sample	Goat Proportion	Sheep Proportion
Hassan	Belur	Hulikere	160	0	160	17	17	0
Hassan	Channarayapatna	Byaladakere	142	91	233	17	10	7
Hassan	Arkalgud	Doddabemmathi	263	4	267	17	17	0
Hassan	Arsikere	CHIKKANANAKOPPLU	102	309	411	17	4	13
Hassan	Hassan	Veerapura	332	1443	1775	17	3	14
Hassan	Hassan	Doddabaganahalli	428	3526	3954	18	2	16
Haveri	Hirekerur	Neshwi	91	341	432	17	4	13
Haveri	Hangal	Shivapura	216	1842	2058	17	2	15
Kodagu	Virajpet	Hebbale	150	0	150	17	17	0
Kodagu	Somvarpet	Thorenur	70	102	172	17	7	10
Kolar	Robertson Pet	Robertson Pet (CMC) - Ward No.20	216	2	218	17	17	0
Kolar	Mulbagal	H.Byappalli	82	283	365	17	4	13
Kolar	Kolar	Marjenahalli	121	387	508	17	4	13
Kolar	Srinivaspur	Hebbata	142	486	628	17	4	13
Kolar	Srinivaspur	M.Thummalappalli	131	502	633	17	4	13
Koppal	Kushtagi	Kodatgeri	196	784	980	17	3	14
Koppal	Kushtagi	Nidsesi	528	1410	1938	17	5	12
Mandya	Maddur	Huligerepura	111	264	375	17	5	12
Mandya	Nagamangala	Manchenahalli	181	319	500	17	6	11
Mandya	Maddur	Malagaranahalli	235	273	508	17	8	9
Mandya	Maddur	Sompura	277	469	746	17	6	11
Mandya	Mandya	Holalu	384	367	751	17	9	8
Mysore	Hunsur	Yamagumbha	184	21	205	17	15	2
Mysore	Nanjangud	Yelachagere	96	335	431	17	4	13
Mysore	Tirumakudal - Narsipur	Chandahalli	209	242	451	17	8	9
Mysore	Heggadadevankote	Rajegowdanahundi	500	116	616	17	14	3
Mysore	Nanjangud	Saragooru	222	527	749	17	5	12
Raichur	Manvi	Harvi	328	513	841	17	7	10
Raichur	Sindhur	Belgurki	579	1041	1620	17	6	11
Ramanagara	Magadi	Bettahalli	271	170	441	17	10	7
Ramanagara	Channapatna	Nunnuru	577	137	714	17	14	3
Shimoga	Shikaripura	Mallenahalli	148	9	157	17	16	1
Shimoga	Bhadravati	Arahatholalu	90	108	198	17	8	9
Shimoga	Shikaripura	Kenchigondanakoppa	157	46	203	17	13	4
Shimoga	Sorab	Kathuvalli	315	27	342	17	16	1
Shimoga	Shikaripura	Kittadahalli	53	390	443	17	2	15
Tumkur	Chiknayakanhalli	Kodalagara	93	36	129	17	12	5
Tumkur	Gubbi	Benchagere	146	124	270	17	9	8
Tumkur	Chiknayakanhalli	Barakanahalu	468	0	468	17	17	0
Tumkur	Tiptur	Shivara	290	351	641	17	8	9
Tumkur	Madhugiri	Chikkamalur	102	651	753	17	2	15
Tumkur	Kunigal	Besinele	254	903	1157	17	4	13
Tumkur	Sira	Devagondanahalli	144	1324	1468	17	2	15
Tumkur	Pavagada	Thimmammanahalli	312	2689	3001	18	2	16
Uttar Kannad	Yellapur	Madnur	8	123	131	17	1	16
Uttar Kannad	Sirsi	Kuppagadde	134	30	164	17	14	3
Uttar Kannad	Mundgod	Agadi	144	85	229	17	11	6
Uttar Kannad	Mundgod	Wadagatta	110	132	242	17	8	9
Uttar Kannad	Mundgod	Katur	245	18	263	17	16	1
Yadgir	Shorapur	Horatti	464	579	1043	17	8	9
Yadgir	Yadgir	Chaler	302	2768	3070	18	2	16
		<b>Total</b>	<b>34420</b>	<b>78153</b>	<b>112573</b>	<b>1588</b>	<b>751</b>	<b>837</b>

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## Authorship Contribution Statement

Suresh K.P: Sampling plan generation and methodology development, Patil S.S: Review of sampling plan, Hemadri D: Review of sampling plan, Raveendra Hegde: Data generation, verification of document and Review of sampling plan, Dharanesh N.K: Data generation, verification of document and Review of sampling plan.



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