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Sampling Plan for Serosurveillance of FMD in Small Ruminants in Karnataka under National Animal Disease Control Programme

(NADCP) - 2023

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

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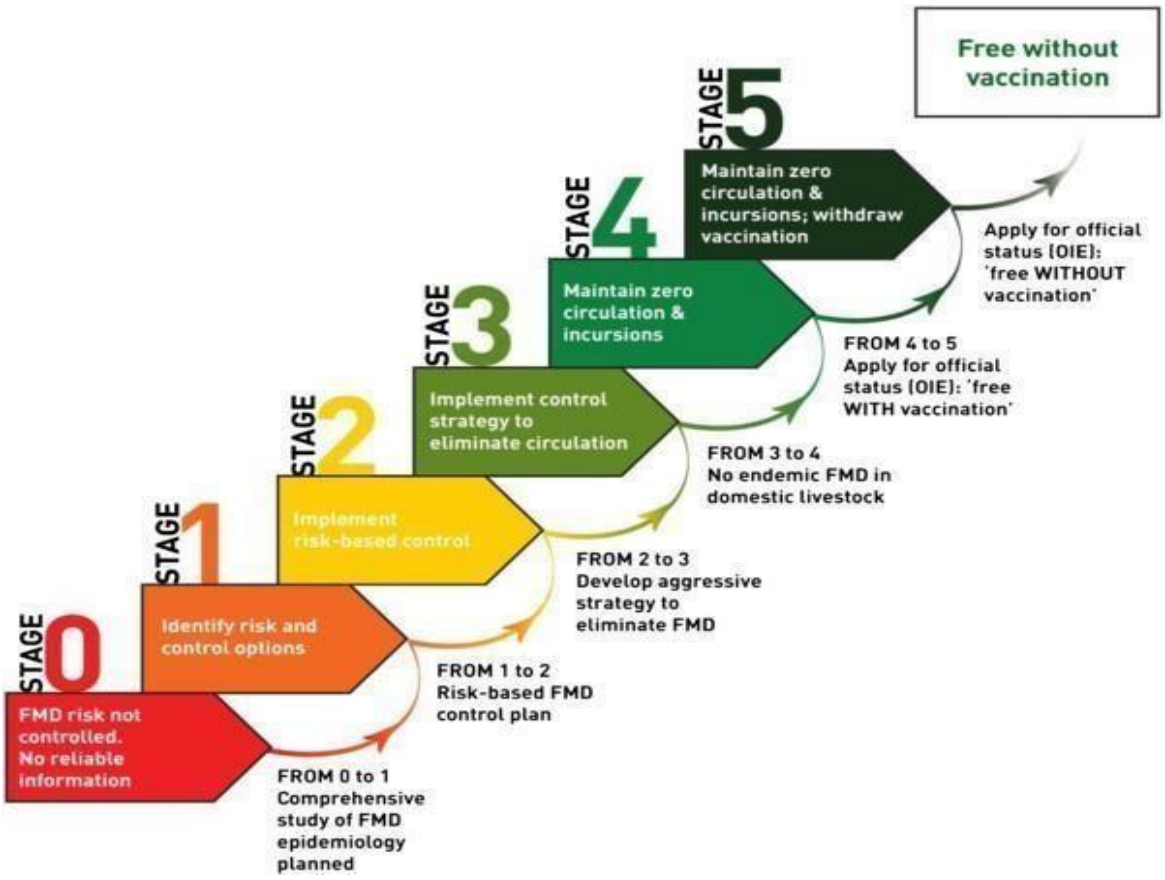
Free without vaccination
STAGE 5
Maintain zero circulation & incursions; withdraw vaccination
Apply for official status (OIE): 'free WITHOUT vaccination'
STAGE 4
Maintain zero circulation & incursions
FROM 4 to 5
Apply for official status (OIE): 'free WITH vaccination'
STAGE 3
Implement control strategy to eliminate circulation
FROM 3 to 4
No endemic FMD in domestic livestock
STAGE 2
Implement risk-based control
FROM 2 to 3
Develop aggressive strategy to eliminate FMD
STAGE 1
Identify risk and control options
FROM 1 to 2
Risk-based FMD control plan
STAGE 0
FMD risk not controlled. No reliable information
FROM 0 to 1
Comprehensive study of FMD epidemiology planned

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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 becausethe 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 presenceof 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 (firststage), and individual animals within selected villages make secondary sampling units (second stage). Inthis way, the sample sizes at each stage can be adjusted to reflect the different disease prevalence's (the proportion of villagesaffected 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 atthe 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
b. Theoretical village level prevalence of 20%, 15%, 10% and 5% for small (states having target population up to 1.5% of total), medium (states having target population up to 5% of total), large (states having target population up to 10 % of total), very large (states having target population more than 10% of total) states respectively to provide better representation of villages within the district and state.
c. diagnostic sensitivity of 60% and specificity of 100% for 3B3 NSP ELISA

Two -stage stratified random sampling plan was generated at reasonably high confidence (0.95%) using in-house developed using epi-calculator under NADRES v2 by ICAR-NIVEDI, Bengaluru.(https://nivedi.res.in/Nadres_v2/Epical/stratified/random_sampling.php). The summary of sampling plan generated using a two-stage stratified random sampling scheme is presented in Tables 1 &2.

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

STATE	NO OF DISTRICTS	NO OF BLOCKS	Total no of Sheep + Goat **	No of Animals to be Sampled	Average No of Samples Per District
Karnataka	29	114	142944	1026	35

**** 20th Livestock Census (DAHD, GoI)** *Animals within 6-18 months of age must be sampled.*
Note: Samples are to be collected preferably within 1-2 months before vaccination or 4-5 months aftervaccination

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

Sl No	*District Names*	*No of Blocks (to be sampled)*	*Total no of population (Sheep + Goat) ***	*Total No of Sheep and Goat population in the sampled villages*	*Total no of samples to beDrawn*
1	*Bagalkot*	*2*	*1006782*	*6925*	*18*
2	*Bangalore Rural*	*3*	*213944*	*1221*	*27*
3	*Belgaum*	*8*	*1459420*	*17459*	*72*
4	*Bellary*	*5*	*1258684*	*17227*	*45*
5	*Bengaluru Urban*	*3*	*145337*	*1481*	*27*
6	*Bidar*	*3*	*268802*	*3043*	*27*
7	*Bijapur*	*2*	*916168*	*5149*	*18*
8	*Chamarajanagar*	*3*	*279954*	*1969*	*27*
P9	*Chikballapur*	*6*	*801585*	*4213*	*54*
10	*Chikmagalur*	*3*	*139002*	*4832*	*27*
11	*Chitradurga*	*3*	*1737145*	*10382*	*27*
12	*Dakshin Kannad*	*2*	*32504*	*827*	*18*
13	*Davangere*	*2*	*630172*	*2821*	*18*
14	*Dharwad*	*2*	*153938*	*2495*	*18*
15	*Gadag*	*2*	*587555*	*4006*	*18*
16	*Gulbarga*	*5*	*558587*	*3823*	*45*
17	*Hassan*	*7*	*328445*	*3911*	*63*
18	*Haveri*	*3*	*458174*	*4168*	*27*
19	*Kodagu*	*1*	*8253*	*328*	*9*
20	*Kolar*	*6*	*577605*	*4181*	*54*
21	*Koppal*	*2*	*797945*	*3531*	*18*
22	*Mandya*	*5*	*693563*	*3272*	*45*
23	*Mysore*	*5*	*411669*	*3307*	*45*
24	*Raichur*	*5*	*940351*	*8089*	*45*
25	*Ramanagara*	*2*	*278118*	*1087*	*18*
26	*Shimoga*	*7*	*102245*	*2731*	*63*
27	*Tumkur*	*8*	*1717934*	*11972*	*72*
28	*Uttar Kannad*	*7*	*19192*	*4730*	*63*
29	*Yadgir*	*2*	*693940*	*3764*	*18*
	Total	*114*	*17217013*	*142944*	*1026*

Note:

Number of reserved villages =4

Total number of samples in Reserved villages =1026

Total No of samples(Final)= 1026-36-=990

Standard Operating Procedure (SOP) for Collection and Dispatch of Sheep and Goat Serumunder 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. (4ºC).

- ❖ 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 smallamount 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^{0}C or colder freezer. All specimens should remain at -20^{0}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^{0}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:

For any clarifications on SOP and Sampling plan issues, please contact:

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

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

Download The Result Generated on 11/05/2023 03:52:35pm

SRK/SEROSUR/NIVEDI/Karnataka16/Phase II

Stratified Random Sampling:

Species Selected for Stratification = Sheep + Goat

Number of Villages Having 200 + (Sheep + Goat) = 10602

Design Level Prevalence = 0.3

Cluster Level Prevalence = 0.03

Sensitivity of the test used = 0.9

Specificity Level Prevalence = 0.9

Total No of Villages (Clusters) Selected = 110 (+4 Reserved Villages)

Total No of Animals to be Sampled = 990

DISTRICT_NAME	BLOCK_NAME	VILLAGE_NAME	Goats Population	Sheep Population	Sheep + Goat Population	Number of units to sample	Goat Proportion	Sheep Proportion	
Bagalkot	Jamkhandi	Gadyal	1961	534	2495	9	7	2	
Bagalkot	Bagalkot	BENNUR PU.K	1695	2735	4430	9	3	6	
Bangalore Rural	Nelamangala	Yeramanchanahalli	308	0	308	9	9	0	
Bangalore Rural	Devanahalli	Thylagere	210	221	431	9	4	5	
Bangalore Rural	Devanahalli	Dodda Thattamangala	84	398	482	9	2	7	
Belgaum	Mudalgi	Mudalgi (TMC) - Ward No.19	178	25	203	9	8	1	
Belgaum	Ramdurg	Haletoragal	565	7	572	9	9	0	
Belgaum	Athni	Khilegaon	860	10	870	9	9	0	
Belgaum	Athni	Bedarahatti	930	577	1507	9	6	3	
Belgaum	Gokak	Patagundi	1441	109	1550	9	8	1	
Belgaum*	Ramdurg	Chiktadashi	702	1215	1917	9	3	6	Reserved
Belgaum	Raybag	Biranal	828	1295	2123	9	4	5	
Belgaum	Raybag	Hubbarwadi	2760	5957	8717	9	3	6	
Bellary	Hagaribommanahalli	MAGIMAVINAHALLI	213	601	814	9	2	7	
Bellary	Kudligi	J.B.HALLI	439	810	1249	9	3	6	
Bellary	Bellary	Chellagurki	365	1368	1733	9	2	7	
Bellary	Kudligi	DODDA GOLLARA HATTI	60	3145	3205	9	0	9	
Bellary	Sandur	C.GOLLARAHATTI	1807	8419	10226	9	2	7	
Bengaluru Urban	JIGANI	Jigani (TMC) - Ward No.1	55	286	341	9	1	8	
Bengaluru Urban	Bangalore South	Bangalore (M Corp.) - Ward No.139	224	240	464	9	4	5	
Bengaluru Urban	Anekal	Singena Agrahara	75	601	676	9	1	8	
Bidar	Homnabad	Nagankera	533	237	770	9	6	3	
Bidar	Bidar	Vilaspur	588	313	901	9	6	3	
Bidar	Bidar	Aliamber	1051	321	1372	9	7	2	
Bijapur	Sindgi	Sindgi (Rural) *	847	621	1468	9	5	4	
Bijapur	Bijapur	Shivanagi	2576	1105	3681	9	6	3	
Chamarajanagar	Yelandur	Kinakahalli	0	430	430	9	0	9	
Chamarajanagar	Chamarajanagar	Bedarapura	323	366	689	9	4	5	
Chamarajanagar	Chamarajanagar	CHUNGADIPURA	332	518	850	9	4	5	
Chikballapur	Gudibanda	Yelakalarallahalli	131	126	257	9	5	4	
Chikballapur	Bagepalli	Kollavaripalli	168	265	433	9	3	6	
Chikballapur	Chintamani	YAGAVA MINDIGAL	124	455	579	9	2	7	
Chikballapur*	Chintamani	Appasanihalli	40	571	611	9	1	8	Reserved
Chikballapur	Bagepalli	Mallegurke	73	988	1061	9	1	8	
Chikballapur	Sidlaghatta	Kyasagere	52	1220	1272	9	0	9	
Chikmagalur	Tarikere	Karkuchi	159	582	741	9	2	7	
Chikmagalur	Kadur	Biluvala	271	959	1230	9	2	7	
Chikmagalur	Kadur	Muthanagere	277	2584	2861	9	1	8	
Chitradurga	Challakere	Matasamudra	500	1510	2010	9	2	7	
Chitradurga	Hiriyur	Gorladaku	608	2857	3465	9	2	7	
Chitradurga	Molakalmuru	Hanagal	3449	1458	4907	9	6	3	
Dakshin Kannad	Puttur	Nelyadi	359	0	359	9	9	0	
Dakshin Kannad	Bantval	PUDU	468	0	468	9	9	0	
Davangere	Malebennur	Malebennur (TMC) - Ward No.7	123	685	808	9	1	8	
Davangere	Harihar	Belludi	494	1519	2013	9	2	7	
Dharwad	Hubli	Ingalhalli	83	508	591	9	1	8	
Dharwad	Navalgund	Gummagol	641	1263	1904	9	3	6	
Gadag	Nargund	Surkod	386	1014	1400	9	2	7	
Gadag	Ron	Yavagala	1132	1474	2606	9	4	5	
Gulbarga	Gulbarga	Kesratgi	491	0	491	9	9	0	
Gulbarga	Gulbarga	Dinshikurnur	555	0	555	9	9	0	
Gulbarga	Jevargi	Kummanasirasgi	466	156	622	9	7	2	
Gulbarga	Sedam	Kanagadda	345	569	914	9	3	6	
Gulbarga	Chincholi	Keroli	1241	0	1241	9	9	0	

Hassan	Channarayapatna	Channenahalli (Channahalli)	79	140	219	9	3	6	
Hassan	Hassan	Markuli	229	132	361	9	6	3	
Hassan	Hole Narsipur	Chikkchagahalli	167	216	383	9	4	5	
Hassan	Hole Narsipur	Anekannambadi	125	435	560	9	2	7	
Hassan	Arsikere	Kondavagilu	140	602	742	9	2	7	
Hassan	Hassan	Hongere	144	640	784	9	2	7	
Hassan	Channarayapatna	Dadigatta	512	350	862	9	5	4	
Haveri	Savanur	Hiremaralihalli	143	401	544	9	2	7	
Haveri	Hangal	Masankatti	96	923	1019	9	1	8	
Haveri	Hirekerur	HADE	365	2240	2605	9	1	8	
Kodagu	Somvarpet	Kudumangalore	304	24	328	9	8	1	
Kolar	Kolar	Kolar (CMC) - Ward No.1	36	188	224	9	1	8	
Kolar	Kolar	Ammerahalli	58	264	322	9	2	7	
Kolar	Srinivaspur	Balthmari	84	465	549	9	1	8	
Kolar	Bangarapet	Mugalabele	95	546	641	9	1	8	
Kolar	Mulbagal	Tayalur	24	900	924	9	0	9	
Kolar	Mulbagal	Bevahalli	32	1489	1521	9	0	9	
Koppal	Kanakagiri	Kanakagiri - Ward No.16	190	1249	1439	9	1	8	
Koppal	Yelbarga	Chendoor	335	1757	2092	9	1	8	
Mandya	Nagamangala	Halthi	139	243	382	9	3	6	
Mandya	Nagamangala	A.Nagathihalli	405	99	504	9	7	2	
Mandya	Nagamangala	Sampanahalli	199	444	643	9	3	6	
Mandya	Mandya	Chikkakothagere	238	592	830	9	3	6	
Mandya	Malavalli	Hullagala	586	327	913	9	6	3	
Mysore	Heggadadevankote	Chakkur	183	251	434	9	4	5	
Mysore	Mysore	Arasinakere	204	271	475	9	4	5	
Mysore	Nanjangud	Hunasanalu	300	329	629	9	4	5	
Mysore	Piriyapatna	N. Settihalli	403	369	772	9	5	4	
Mysore	Nanjangud	Konanoor	375	622	997	9	3	6	
Raichur	Raichur	Chicksugur	192	629	821	9	2	7	
Raichur	Lingsugur	Hosagudda	462	531	993	9	4	5	
Raichur	Devadurga	Hosur Siddapur	434	794	1228	9	3	6	
Raichur	Lingsugur	Mudaldinni	278	1694	1972	9	1	8	
Raichur	Raichur	Merched	567	2508	3075	9	2	7	
Ramanagara	Channapatna	Kukkur	172	300	472	9	3	6	
Ramanagara	Kanakapura	Makkalandha	202	413	615	9	3	6	
Shimoga	Shimoga	Kunchena Halli	143	63	206	9	6	3	
Shimoga	Shivamogga	Shimoga (CMC) - Ward No.5	182	82	264	9	6	3	
Shimoga	Shikaripura	Dabbanabhyranahalli	111	192	303	9	3	6	
Shimoga	Sorab	Chikkabburu	299	8	307	9	9	0	
Shimoga*	Bhadravati	Bhadravati (CMC) - Ward No.23	262	162	424	9	6	3	Reserved
Shimoga	Bhadravati	Singanamane	285	175	460	9	6	3	
Shimoga	Shikaripura	Kappanahalli	294	473	767	9	3	6	
Tumkur	Koratagere	Koratagere (TP) - Ward No.5	243	41	284	9	8	1	
Tumkur	Kunigal	Hulipura	137	235	372	9	3	6	
Tumkur	Koratagere	Bajjanahalli	154	501	655	9	2	7	
Tumkur	Chiknayakanhalli	Chikkaramapura	511	321	832	9	6	3	
Tumkur	Turuvekere	Sampigehosahalli	620	468	1088	9	5	4	
Tumkur	Sira	Panjiganahalli	90	1279	1369	9	1	8	
Tumkur	Pavagada	Thappaganadoddi	131	2020	2151	9	1	8	
Tumkur	Sira	Ganadahunase	632	4589	5221	9	1	8	
Uttar Kannad	Sirsi	Badanagod	154	114	268	9	5	4	
Uttar Kannad	Haliyal	Ghadiyal	231	57	288	9	7	2	
Uttar Kannad	Mundgod	Hungunda	402	64	466	9	8	1	
Uttar Kannad	Mundgod	Indoor	135	439	574	9	2	7	
Uttar Kannad*	Haliyal	Jatga	801	29	830	9	9	0	Reserved
Uttar Kannad	Mundgod	Kopp (Ind)	86	806	892	9	1	8	
Uttar Kannad	Mundgod	Bachanaki	556	856	1412	9	4	5	
Yadgir	Shahpur	Hattigudur	814	943	1757	9	4	5	
Yadgir	Yadgir	Karengi	194	1813	2007	9	1	8	

*Reserved villages to be used for sampling if any selected village in given district is not accessible, having logistic probelm or any other issues

Unreserved Villages 110	**990**
Reserved Villages 4	**36**
Total samples	**1036**

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