Evidence Action

Deworm the World Initiative

Prevalence of Soil Transmitted Helminths in the State of Rajasthan

Summary Report

Prepared by Deworm the World Initiative, January 23, 2014

Submitted to: Department of Education, Government of Rajasthan; Department of Medical, Health and Family Welfare, Government of Rajasthan.

Special appreciation to*: National Institute of Cholera and Enteric Disease, Kolkata , an institute of Indian Council of Medical Research for undertaking the Laboratory Analysis ; University of California, Berkeley for sample framing and guidance in data analysis; and The Network for Engineering and Economics Research NEERMAN, Mumbai, for undertaking the exhaustive field work, data analysis, report preparation, and for coordination with all the collaborating institutes.*

EXECUTIVE SUMMARY

The World Health Organization (WHO) estimates that 1.5 billion people worldwide are infected with soil transmitted helminths (STH) and over 870 million of those infected are preschool and school-age children. STH infections can have significant impacts on the health and educational outcomes of these children. In light of high rates of prevalence found in other parts of India, the Government of Rajasthan requested **Deworm the World Initiative** (DtWI) to investigate the prevalence of STH in Rajasthan.

At the request of the Government of Rajasthan, DtWI conducted a prevalence survey in Rajasthan. Laboratory analysis was conducted by the National Institute of Cholera and Enteric Diseases (NICED). The non-profit group, Network for Engineering and Economics Research (NEERMAN), provided field staff, undertook sample collection, and conducted data analysis. Guidance for the sampling design and analysis of the data was provided by the University of California, Berkeley, with inputs from DtWI and NEERMAN. To represent the varying environmental characteristics of the state, 144 schools from 12 districts and 36 blocks were randomly chosen from across the state using multi-stage probability proportional to size sampling. From these 144 schools, 2,635 samples were collected from school children. These samples were preserved using SAF solution (a mixture of formaline, distilled water, sodium hypochloride solution and glacial acetic acid), and sent by cold chain to a NICED laboratory in Kolkata. NICED analyzed the samples using Kato Katz methodology to determine the prevalence of each type of STH, and certified the results presented here.

Average prevalence of any STH in the state was found to be 21.1%. Average statewide prevalence of Ascaris, Hookworm, and Trichuris was found to be 20.2%, 1%, and 0.2% respectively. On the basis of WHO guidelines (http://whqlibdoc.who.int/publications/2011/9789241548267_eng.pdf) which recommend annual mass deworming in areas with STH prevalence between 20% and 50%, DtWI recommends an annual mass deworming in Rajasthan.

SITUATION ANALYSIS

The soil-transmitted helminths (STH) - roundworm, whipworm, and hookworm - are widespread in the tropical and subtropical parts of the developing world where there are inadequate sanitation facilities and clean water. WHO estimates that more than 1.5 billion people, or 24% of the world's population, are infected with STH[1], with over 241 million school-age or younger children in India at risk and requiring treatment. STH infection can lead to anemia, malnutrition, impaired mental and physical development, and reduced school participation. Thus, the WHO recommends that regions with higher than 20% prevalence of STH implement mass deworming[2]. These WHO guidelines are presented in Table 1 on the following page.

Previous studies in different parts of India have indicated that STH prevalence is a problem in India. The Government of Rajasthan has recognized the significant public health impact of STH infections in children under the age of 14. A 2012 study conducted by Panchayati Raj Department, Government of Rajasthan, with support from UNICEF, identified only 27.3% of households with a toilet, and high levels of open defecation among the households ranging from 54.3% in Bikaner to 88.4% in Dhaulpur[3]. Given these sanitation indicators, it was expected that STH infection would be a public health problem in Rajasthan. However, no reliable scientific data was available on the prevalence of STH in the state.

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[1] http://www.who.int/mediacentre/factsheets/fs366/en/

[2] "Helminth control in school-age children: A guide for managers of control programmes", Second edition, World Health Organization, 2011, http://whqlibdoc.who.int/publications/2011/9789241548267_eng.pdf

[3] "WASH Validation in Rajasthan." UNICEF Rajasthan and Government of Rajasthan, 1/29/13.

Therefore, the government requested technical support from **Deworm the World Initiative (DtWI)** to conduct a field survey to estimate STH prevalence. A prevalence survey is a scientifically designed study intended to measure the extent to which STH are endemic in a given population. Stool samples are collected from an appropriately weighted and representative sample from the state, analyzed by laboratory technicians for presence of STH according to standardized methods, and the subsequent data is used to arrive at statewide prevalence estimates for each and any STH. The following report details this prevalence survey and the results that came out of this analysis.

Table 1: WHO Guidelines on Control Strategies for STH

Category	Prevalence of any STH infection at baseline	Control Strategy	
		Preventive chemotherapy	Additional Interventions
Schools in high-risk areas	≥ 50%	Treat all school-age children (enrolled and non-enrolled) twice a year.	Improve sanitation and water supply. Provide health education.
Schools in low-risk areas	≥ 20% and < 50%	Treat all school-age children (enrolled and non-enrolled) once a year.	Improve sanitation and water supply. Provide health education.

STUDY DESIGN

A state level survey was designed to estimate STH prevalence among the children enrolled in primary schools across the state. This study was designed with assistance from Professors Jack Colford and Ben Arnold at the University of California, Berkeley. Ethical clearance for the study to collect the samples was taken from an Indian independent ethics committee, Suraksha Independent Ethics Committee (SIEC). The study was approved as per Good Clinical Practice guidelines for biomedical research on Human Subjects, issued by Indian Council of Medical Research (ICMR). The sample frame consisted of all primary schools in the state which had at least 15 enrolled students. The sample of 144 schools was selected using a multi-stage probability proportional to size (PPS) sampling strategy to ensure that the sample was truly random and representative. The sample was selected such that it represented the main ecological zones in Rajasthan and designed to give a statewide prevalence of STH. The number of districts, blocks, schools, and children sampled are described in Table 2.

Table 2: Sample Frame

Number of districts selected in the sample	12
Number of blocks selected from each district	3 (12 districts x 3 blocks) = 36 blocks
Number of schools from each block	4 (36 blocks x 4 schools) = 144 schools
Number of children from each school to be given pots	30 (144 schools x 30 children) = 4320 children
Assuming a 50% return rate, total sample size	50% of 4320 = 2160 children

Assuming a required precision on the prevalence estimate of +/- 3% and taking into account the multi-stage clustered design (with a design effect estimated at 3 based on a previous survey), the required sample size for this study was 2049 students. In addition, a socio-economic questionnaire was developed to ensure that the households from which samples were collected were representative and there was no bias in the sample collection process.

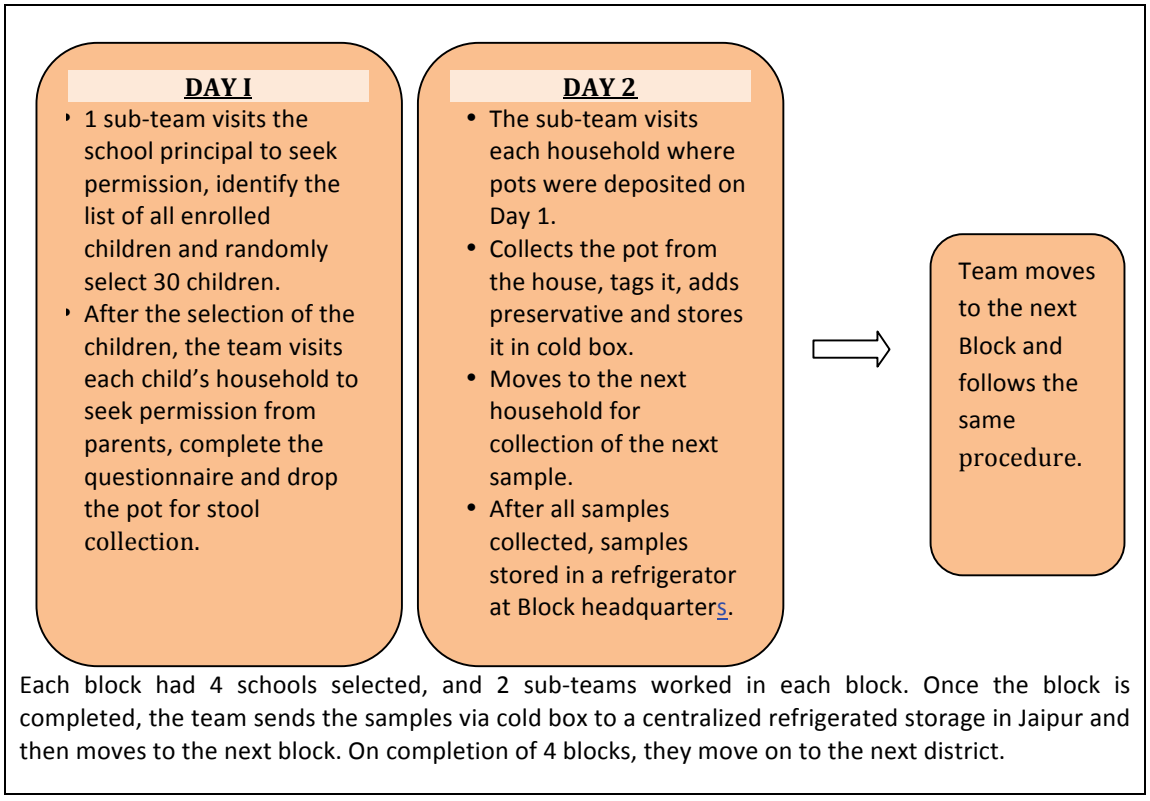
FIELD WORK

The field work consisted of two stages: a training stage, where field personnel were trained to collect and store samples, and a sample collection stage, where field teams collected samples from households around Rajasthan. The details of the two stages are as follows:

Training: Training for the field teams was conducted in Rajasthan, by personnel from NICED, NEERMAN, and DtWI. These field teams had prior experience with stool collection surveys in other parts of India. Training focused on the methodology for administering the socio-economic questionnaire, field conduct, contingency measures, sample selection of children, personal and respondent safety, informed consent, and collection, labeling, storage, and transportation of samples. The training format included rigorous classroom lectures, question and answer sessions, mock interviews, and field practice.

Sample collection: The fieldwork consisted of collection of stool samples from the selected children, preserving the samples using a SAF solution (a mixture of formaline, distilled water, sodium hypochloride solution and glacial acetic acid), and then maintaining samples in a cold chain all the way to NICED in Kolkata. To undertake the task, 6 field teams were formed, each constituted of 4 enumerators and 1 supervisor. The field plan flow in every block is depicted in Figure 1.

Figure 1: Field Plan Flow



Samples were collected from 2,635 households in this way, exceeding the target of 2,049 samples required to give statewide prevalence. Household return rates were thus higher than the expected 50%.

LABORATORY ANALYSIS

The stool specimens were transported to NICED, an Institute Council of Medical Research Institute in Kolkata, using cold boxes. Upon arrival the samples were stored in a 4°C chamber and were later examined for any problems by NICED technicians. Any samples which indicated spillage, loss of

preservative, or any other issue potentially affecting the analysis were rejected. A total of **2075** samples were found suitable for testing and consequently tested in the NICED laboratory. NICED used a modified Kato-Katz (KK) method to detect STH infections (the KK method is recommended by the WHO[4] and the method used here is described in **Annexure 1)**. The method was modified to work with SAF preservation, using a concentration method for the stool aliquots that significantly increased the test sensitivity (higher likelihood of detecting low intensity infections). The protocol followed by NICED is attached as **Annexure I** and the Certificate of Testing from NICED is attached as **Annexure II.** All the results for the tested samples were entered into Stata for data analysis.

DATA ANALYSIS

In order to ensure that the tested samples were truly random and did not exhibit any bias, probability proportional to size sampling had been used in the design of the sample. However, since some samples were not collected (because selected households refused to give a sample) and some collected samples were not tested (since some samples were rejected), it was necessary to check whether there were any biases in the samples collected and analyzed. To check for this bias, we looked at whether the key socio-economic and demographic characteristics were different between the children whose stool samples were tested and compared them to the children whose stool samples were either not collected or rejected. The logistic regression models used in this assessment did not uncover any significant imbalance. Had there been any imbalance, then we would need to further analytical adjustments to the estimated prevalence to reduce the bias in estimated prevalence.

To get more accurate point estimate of the prevalence we weight each observation (test result) by inverse of the survey response rate (ratio of the number of samples tested to the number of children surveyed) for the corresponding school. Because our sample was PPS, it is self-weighting and we did not adjust for different probability of selection for children in our sample. We used the *means* command in STATA to estimate prevalence. In order to graphically demonstrate the likely prevalence of STH across the entire state, we used spatial extrapolation tools available in ArcGIS.

QUALITY ASSURANCE:

Field Level Quality Assurance: One supervisor was responsible for two sub teams of field workers. The supervisor accompanied each of the two sub teams on alternate days and was responsible for logistical planning, trouble-shooting, and maintenance of log of field work, as well as monitoring the storage and preservation of samples between villages and blocks. These supervisors further reported their day-to-day activities to two senior supervisors. For spot checks, monitoring and supervision staff from NEERMAN and DtWI also visited the field teams every week during the collection period.

Senior supervisors re-visited approximately 20% of the schools to check if the field workers were visiting the selected schools and to ensure that children were selected as per random list provided to them. Approximately 10% of the surveyed households were also checked to confirm that the field workers had collected samples from the appropriate household and to confirm the identification of the sample.

Laboratory Quality Assurance: Samples were received by senior NICED researchers and were immediately sent to cold storage. After microscopic analysis using the Kato Katz method established the presence of STH, all positive samples were rechecked by a microbiologist to ensure accuracy. 10% of the negative samples were randomly chosen and rechecked to minimize false negative readings.

[4] "Helminth control in school-age children: A guide for managers of control programmes", Second edition, World Health Organization, 2011, http://whqlibdoc.who.int/publications/2011/9789241548267_eng.pdf.

Additionally, during the laboratory analysis of the samples, a researcher from DtWI visited the NICED laboratory to monitor sample storage, slide preparation, reading of slides, preservation of slides, and the photographic record of the STH prevalence.

RESULTS

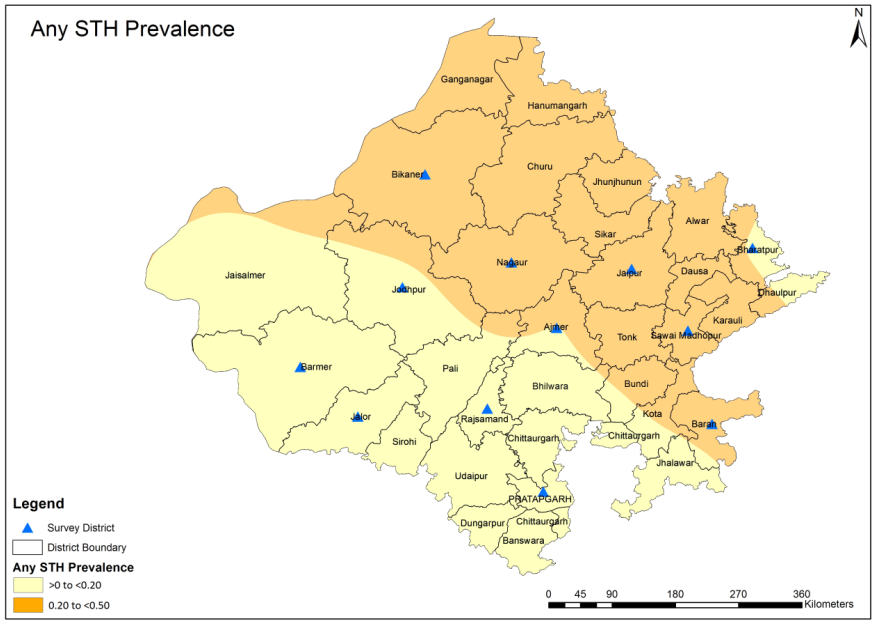
The mean statewide prevalence of any STH (Ascaris, Trichuris or Hookworm) was found to be 21.1%. The prevalence of Ascaris infection was found to be highest of the three STH with an average prevalence of 20.2%. The average prevalence of hookworm was 1% and Trichuris was 0.2%. Pictures of the STH taken during laboratory analysis are attached in **Annexure III**. The state level prevalence of each STH is presented in Table 3.

Table 3: State Level Prevalence of STH

STH	Prevalence
Ascaris	20.2%
Trichuris	0.2%
Hookworm	1%
Any STH	21.1%

The GIS based extrapolation of the prevalence of any STH infection across the state of Rajasthan is provided in Figure 2. Additional state level maps for each STH infection are presented in Annexure IV.

Figure 2: Prevalence of any STH in Rajasthan state



At the district level, the mean estimated Ascaris prevalence ranges from 6.4% in Prataphgarh to 38% in Bikaner. The upper end of the 95% confidence Interval for Ascaris prevalence is at maximum 46.6% (Bikaner). Therefore, with 95% confidence, no district was expected to exceed 50% prevalence. The mean Trichuris prevalence is largest in Jaipur district, at only 2%. The estimated mean hookworm prevalence is highest in Bharatpur (3.6%) and Pratapgarh (3.5%). The district level prevalence of STH in the 12 surveyed districts is presented in Table 4.

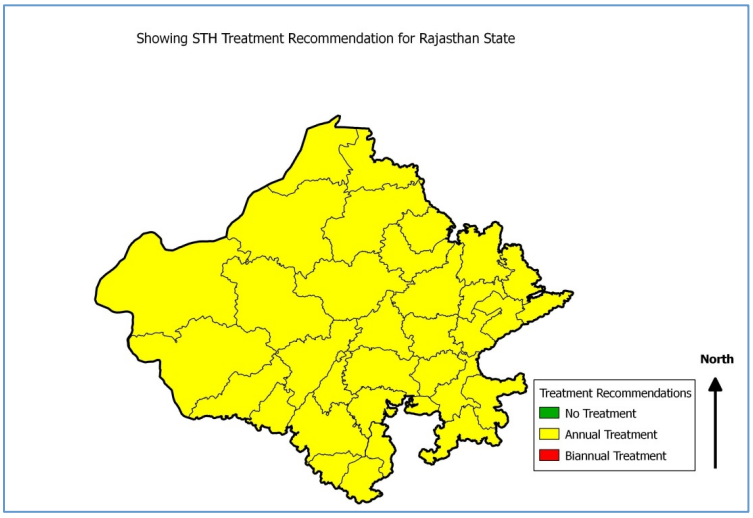
Table 3: District level STH prevalence

District	Prev Ascaris	Prev Trichuris	Prev Hookworm	Prev Any STH
AJMER	18.7%	nil	0.6%	19.4%
BARAN	21.2%	nil	nil	21.2%
BARMER	11.5%	nil	nil	11.5%
BHARATPUR	15.6%	nil	3.6%	19.2%
BIKANER	38%	nil	nil	38%
JAIPUR	35.4%	2%	1.2%	35.9%
JALOR	6.6%	nil	0.8%	7.3%
JODHPUR	14.3%	nil	nil	14.3%
NAGAUR	34.5%	nil	nil	34.5%
PRATAPGARH	6.4%	nil	3.5%	9.9%
RAJSAMAND	14.7%	nil	2%	16.3%
SAWAI MADHOPUR	24.6%	0.6%	0.8%	25.3%

RECOMMENDATION

The main objective of this study was to estimate the state level prevalence of STH in Rajasthan. In order to do so, DtWI partnered with NICED, a WHO collaborating organization, and NEERMAN, a reputable NGO with experience in stool sample collection in a number of different states, to conduct a rigorous prevalence survey in Rajasthan. Samples were taken from 2,075 randomly selected households across Rajasthan, taking into account the different ecological zones in the state, and tested for STH. NICED certified that testing of the samples met the highest scientific standards. The subsequent data analysis indicated the average statewide prevalence of STH to be 21.1%. Prevalence at the district levels were also assessed, but no area with greater than 50% prevalence was identified. WHO guidelines recommend that an annual mass deworming program be conducted in areas where prevalence falls in the range of 20% to 50% (WHO, 2011). Since the prevalence of STH in Rajasthan falls within this range, it is recommended that Rajasthan continue conduct an annual mass deworming program in the state. This annual treatment will ensure that the Government of Rajasthan is able to cost effectively combat STH prevalence in the state, meeting WHO treatment recommendations, and allow it to direct saved resources to additional health and sanitation programs in the state.

Figure 3: Treatment Recommendation for Rajasthan



ANNEXURE I: LABORATORY PROTOCOL

Upon arrival the samples were immediately transferred to 4°C chamber for proper preservation.

As the samples were already mixed with SAF preservative a modified Kato-Katz were performed. This method increases the chance of getting helminth eggs in the stool sample even with a gap of few days between sample collection and detection.

**Note: The epg (egg per gram) count obtained from this protocol does not reflect the actual count.

1. The samples were first poured into a 50ml conical tube and were stirred well with the help of an applicator stick so that the big stool masses are broken and the eggs become free from the stool debris.
2. Then the samples were centrifuged at 1800rpm for 10mins in the same tube so that the eggs settle down at the bottom and the preservative can be easily separated by discarding the supernatant (Figure A1).

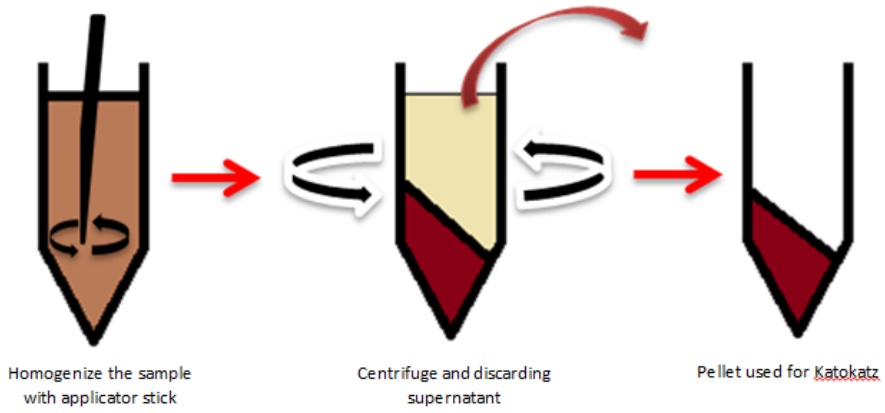


Figure A1. Preparation of stool sample for Kato-Katz test at the laboratory

3. The stool pellets containing the eggs were used for the Kato-Katz preparation following the kit protocol.

Kato-Katz Procedure

1. Place a small amount of fecal material on the newspaper or scrap paper and press a piece of nylon screen on top so that some of the feces are sieved through the screen and accumulated on top.
2. Scrap the flat-sided spatula across the upper surface to collect the sieved feces.
3. Place the template on the centre of the microscope slide and add feces from the spatula so that the hole is completely filled.
4. Pass over the template using the side of the spatula to remove excess feces from the edge of the hole (the spatula and template may be discarded or reused if carefully washed).
5. Remove the template carefully so that the cylinder of feces is left on the slide.
6. Cover the fecal material with a pre-soaked cellophane strip; the strip must be very wet if the feces are dry and less if the feces are soft (if excess glycerol solution is present on the upper surface of the cellophane, wipe it with toilet paper). In dry climates, excess glycerol will retard but not prevent drying.

7. Invert the microscope slide and press the fecal sample against the hydrophilic cellophane strip on another microscope slide or on a smooth hard surface, such as a piece of tile or a flat stone. The fecal material will be spread evenly between the microscope slide and the cellophane strip. It should be possible to read a newspaper print through the smear after clarification.
8. Carefully remove the slide by gently sliding it sideways to avoid separating the cellophane strip or lifting it off. Place the slide on the bench with the cellophane upwards. Water evaporates while the glycerol clears the feces.
9. For all except hookworm eggs, keep slide for one or more hours at room temperature to clear the fecal material, prior to examination under the microscope. To speed up clearing, the slide can be placed in a 40^0 C incubator or kept in direct sunlight for several minutes.
10. Ascaris lumbricoides and Trichuris trichiura eggs will remain visible and recognizable for many months in these preparations. Hookworm eggs clear rapidly and will no longer be visible after 30-60 minutes.
11. The smear should be examined in a systematic manner and the number of eggs of each species reported. Later, multiply this number by 24 to obtain the number of eggs per gram of feces (epg).

ANNEXURE II: (CERTIFICATE OF TESTING FROM NICED)

राष्ट्रीय कॉलरा और आंत्र रोग संस्थान
(भारतीय आयुर्विज्ञान अनुसंधान परिषद्)

NATIONAL INSTITUTE OF CHOLERA AND ENTERIC DISEASES
(INDIAN COUNCIL OF MEDICAL RESEARCH)

WHO COLLABORATING CENTRE FOR RESEARCH AND TRAINING ON DIARRHOEAL DISEASES
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To whom it may concern

As per our ongoing project with NEERMAN we have helped them in detection of soil transmitted helminth from stool samples. The study was a part of pre-deworming STH prevalence survey of primary school children in Rajasthan State. We received stool samples from 2635 children and tested 2075 samples that were found suitable for testing. We have used a modified Kato katz method for screening of stool samples for helminth egg detection. The method used was validated through a pilot study and was found to be effective for current setting as a developing country.

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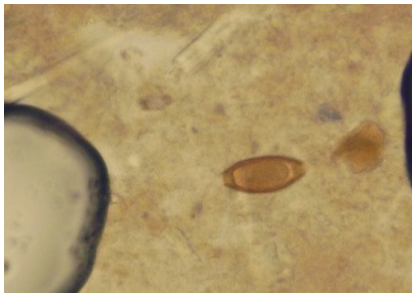
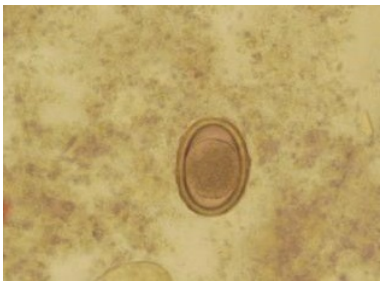
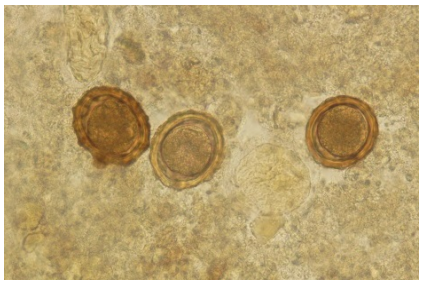
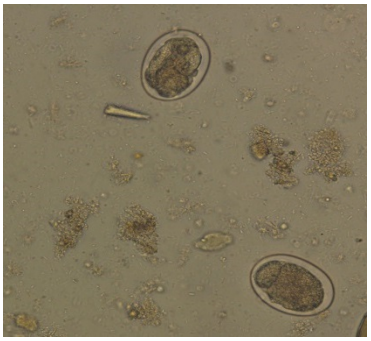
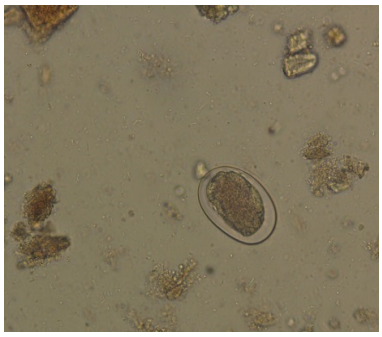
ANNEXURE III: SAMPLE PICTURES OF THE HELMINTH EGGS FOUND IN THE STUDY

Trichuris trichiura

Ascaris lumbricoide

Hookworm

ANNEXURE IV: GIS MAPS FOR STATE LEVEL PREVALENCE FOR ASCARIS, HOOKWORM AND TRICHURIS

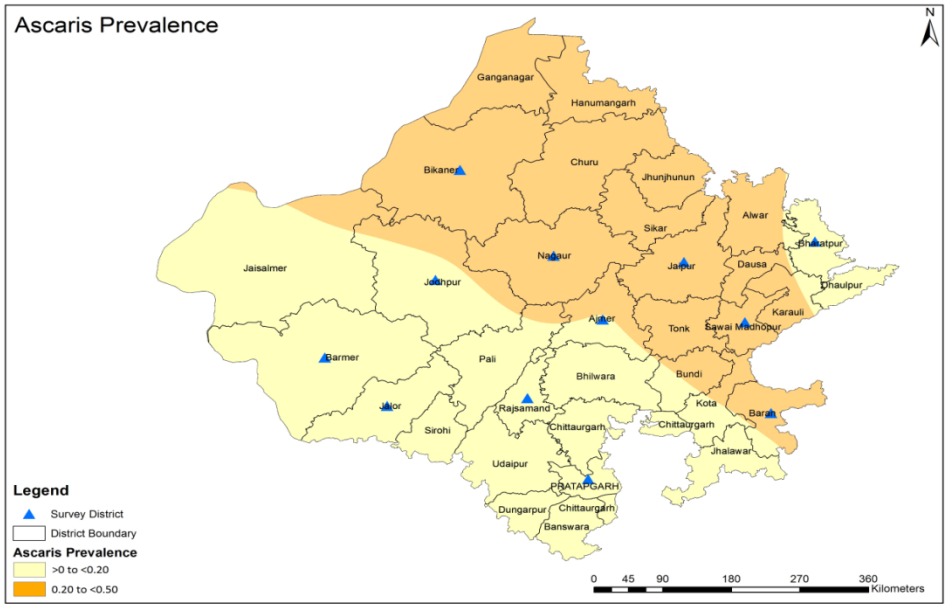


Fig A1: Prevalence of Ascaris at State level

Fig A2: Prevalence of Hookworm at State level

Fig A3: Prevalence of Trichuris at State level