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The "REACH-Bhutan" study: cervical cancer screening in rural Bhutan

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Title: The "REACH-Bhutan" study: cervical cancer screening in rural Bhutan

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Brief title: Cervical cancer screening in rural Bhutan

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Abstract (words=298)

Objectives. The Bhutanese Screening Programme recommends a Pap smear every three years for women aged 25–65 years and coverage ranges from 20% to 60%, being especially challenging in rural settings. The "REACH-Bhutan" study was conducted to assess the feasibility and outcomes of a novel approach to cervical cancer screening in rural Bhutan.

Design. Cross-sectional, population-based, study of cervical cancer screening based on the careHPV test on self-collected samples.

Setting. Women were recruited in rural primary health care centres, i.e. Basic Health Units (BHU), across Bhutan.

Participants. Overall, 3,648 women aged 30–60 years were invited from 15 BHUs differing in accessibility, size, and ethnic composition of the population.

Interventions. Participants provided a self-collected cervico-vaginal sample and were interviewed. Samples were tested using *care*HPV in Thimphu (the Bhutanese capital) referral laboratory.

Main outcome measures. Screening participation by geographic area, centre, age, and travelling time. Previous screening history and *care*HPV-positivity by selected characteristics of the participants.

Results. In April/May 2016, 2590 women (median age: 41 years) were enrolled. Study participation was 71% and significantly heterogeneous by BHU (range: 31%–96%). Participation decreased with increase in age (81% in 30–39 year-old women but only 59% in ≥50 years), and travelling time (90% in women living <30 minutes from the BHU *versus* 62% among those >6 hours away). 50% participants reported a previous screening, the proportion of never-screened women varied significantly by BHU (range: 2%–72%). 265 women (10%; 95%CI 9%–11%)

were *care*HPV-positive, with a significant variation by BHU (range: 5%–19%) and number of sexual partners (prevalence ratio for ≥3 *vs.* 0–1=1.55; 95% CI: 1.05–2.27).

Conclusions:

Community-based cervical cancer screening, testing self-collected samples, can achieve high coverage in rural Bhutan. New solutions to bring self-collection, HPV testing, and precancer treatment to the remotest villages are needed.

Keywords: Cervical cancer screening; self-collection; *care*HPV; rural population; Bhutan.

Article summary section:

Strengths and limitations of this study

- The study was conducted countrywide in a range of rural primary health care centres, which varied in accessibility, size, and ethnic composition of the target population.
- The target population of each centre was enumerated on the basis of up-to-date and detailed demographic surveys.
- A reliable local mobile data network ensured timely and effective study coordination, data collection, and quality controls.
- The proposed diagnostic and treatment solutions presented specific challenges and were less reliable than expected.

INTRODUCTION

Cervical cancer represents the most common cancer among females in Bhutan, with an age-standardised incidence rate of approximately 13 cases per 100,000 person years.[1] The country is strongly engaged in the prevention of cervical cancer. In the year 2000, the Ministry of Health launched a national cytology-based screening programme, and a Pap smear is currently recommended every three years to women aged 25–65 years.[2] In the year 2010, Bhutan was the first low/middle-income country (LMIC) to initiate a successful national vaccination programme against human papillomavirus (HPV) with >90% coverage in girls age 12–18 years.[3] Conversely, screening coverage is much lower and fairly heterogeneous across the country, ranging from 20% to 60% of the target population in different provinces.[4,5] In rural areas, where approximately 60% of the Bhutanese live,[6] cytology-based screening campaigns are occasionally conducted but coverage remains poor,[4] follow-up and treatment of screening-positive women challenging. Organized screening programmes are demanding in terms of administrative, human, financial, and logistic resources. Pap smear-based programmes require frequent visits, trained staff, and strict quality control. In contrast, HPV-based screening allows for longer screening intervals,[7,8] self-sampling,[9] and automation of the diagnostic procedure. It is therefore an attractive option to improve the acceptability and cost-effectiveness of screening.[10]

In 2016, the Ministry of Health of Bhutan and the International Agency for Research on Cancer (IARC) implemented the "REACH-Bhutan" study to assess the feasibility, outcomes, and challenges of cervical cancer screening based on the *care*HPV test on self-collected samples among women 30–60 years of age in rural areas of the country.

MATERIAL AND METHODS

Study population and recruitment

Our study targeted women aged 30–60 years living in rural areas; recruitment and sample collection took place from April to May 2016 in Basic Health Units (BHUs), the facilities that provide primary health care to the Bhutanese population. The Bhutanese principal investigator (UT) selected 15 BHUs that differed by accessibility, size, and ethnic composition of the served population (Figure 1). Lists of household members in each village were obtained from the BHU survey produced yearly for demographic purposes.

Local health workers (HWs) visited the villages served by the selected BHUs to invite eligible women to come to the BHU to participate in the study on specified dates. The benefits of cervical cancer screening and the purposes of the study were explained to the women during public invitation sessions. Women from the same village were invited to attend screening at the BHUs on the same date. Pregnant women, women with mental disability, who had undergone hysterectomy or planned to leave the study area in the next 6 months were not eligible.

On the appointment date, two or three HWs and one of the two mobile study teams (one for East and one for West Bhutan, each composed of two nurses and supervised by a gynaecologist) provided additional information on the study, collected an informed consent form, and administered a short electronic questionnaire on the BHU's premises. The entire process required less than 20 minutes and certain BHUs were able to manage more than 100 women per day. Whenever possible, the recently introduced national citizenship identification number (CID), which uniquely identifies Bhutanese citizens, and at least one mobile telephone number were recorded for follow-up purposes.

Sample collection, transportation, and laboratory analysis

Each participant was asked to provide a self-collected cervico-vaginal sample using a *care*Brush. They were instructed to insert the brush deep into the vagina and rotate it 3 to 5 times before dipping it in a tube containing *care*HPV collection medium. Tubes were then stored in fridges until they were transported in cool boxes to the central laboratory of Jigme Dorji Wangchuck National Referral Hospital (JDWNRH) in Thimphu. Samples were stored at ~4º C and an aliquot was tested using the *care*HPV platform (Qiagen Corporation, Gaithersburg, MD, USA) according to the manufacturer's instructions. The *care*HPV test is a validated signal-amplification, rapid batch diagnostic test for the detection of DNA of 13 high risk (HR) HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and HPV66.[11] *care*HPV results were communicated as soon as possible to each BHU to arrange follow-up of *care*HPV-positive women and of a fraction of *care*HPV-negative women, as a quality control measure.

Data collection and statistical analyses

All information on study participants and biological samples were collected and stored on portable electronic devices. Whenever a mobile data network was accessible, the stored data were uploaded to the IARC server and portable devices were synchronized with the most recent version of the study database. The distribution of the target population in each BHU by age group and by village of residence was obtained from the demographic survey conducted in year 2015. Travelling time from village of residence to the corresponding BHU was estimated for each woman. On account of the socio-geographic characteristics of the study areas, most women had to reach the BHU on foot. We subdivided the travelling time into seven categories (i.e. <30 minutes, 30 to 59 minutes, 1 hour to 1 hour 59 minutes, 2 hours to 2 hours 59 minutes, 3 hours to 3 hours 59 minutes, 4 hours to 5 hours 59 minutes, and 6 or more hours), and fitted a mixed-

effect logistic model to data to assess the influence of travelling time on study participation, with BHUs as clusters.

Among participating women, we assessed prevalence ratios (PR) and corresponding 95% confidence intervals (CI) for lack of previous cervical cancer screening and *care*HPV positivity according to selected characteristics using binomial regression models with a log link and adjustment for age group (30–39, 40–49, and ≥50 years) and BHU of recruitment, as appropriate. For each variable of interest, missing or undisclosed (i.e. labelled as "prefer not to answer") information were treated as not informative and excluded from the analyses.

The outcome of follow-up and clinical management of *care*HPV-positive women will be reported in a future publication.

RESULTS

Out of 15 BHUs included in "REACH-Bhutan", 6 are located in West Bhutan and 9 in East Bhutan (Figure 1). They serve a total of 3,648 women age 30–60 years (Figure 2). Overall participation was 71% (95% CI: 69.5%–72.5%) and was similar in the West and the East. It was, however, significantly heterogeneous by BHU (range: 31%–96%, I-square 99%, p<0.001), and age group (81% in 30–39 year-old women but only 59% in ≥50 years). Participation steadily decreased with the increase in travelling time from village to BHU being 90% (95% CI: 84%–94%) for women living less than 30 minutes from the BHU but 62% (95% CI: 50%–73%) among those 6 hours away or more (see appendix p1). The influence of travelling time strongly increased with a woman's age: the drop in participation between 30 minutes and 6 hours was from 93% (95% CI: 87%–96%) to 76% (95% CI: 64%–84%) among women 30–39 years old, but from 86% (95% CI: 75%–92%) to 45% (95% CI: 30%–62%) among women ≥50 years

(Figure 3). The village of residence was not reported by 6 women or did not belong to the BHU catchment area (7 women). These women did not contribute to the analysis reported in Figure 3.

A total 2,590 women accepted the invitation and came to a BHU (median age: 41 years; inter-quartile range: 35 to 49 years). Ninety-nine percent belonged to 4 ethnic groups: Scharchop (55%); Lhotsampa (27%); Ngalop (13%); and Khengpa (4%), each group predominating in at least one BHU (Figure 1). All but two women provided a telephone number and 60% provided their identity card number. Most women stated that self-sampling was easy to perform (96%) and painless (90%). Macroscopic blood traces were observed in 3·5% of sample of non-menstruating women (data not shown).

Fifty percent of participants reported having had previous screening (Figure 4A) and, among them, 83% had had a Pap smear in the last 4 years (data not shown). The proportion of never-screened women varied across individual BHUs (range: 2%–72%, I2 = 99%, P-value<0.001). Table 1 shows the characteristics that were significantly associated with lack of screening, i.e., age group (PR for ≥50 *vs.* 30–39 years =1.36; 95% CI: 1.26–1.47); region (PR for West *vs.* East=2·84; 95% CI: 2.05–3.95); travelling time from village to BHU (PR for ≥6 hours *vs.* <1 hour =1.53; 95% CI: 1.38–1.69); ethnicity (PR *vs.* Scharchop = 1.65; 95% CI: 1.52–1.80 for Ngalop; 1.41; 95% CI: 1.29–1.53 for Lhotsampa; and 0.58; 95% CI: 0.41–0.81 for Khengpa); educational level (PR for literate *vs.* illiterate =0.85; 95% CI: 0.73–0.99); occupation (PR for shopkeepers/saleswoman/manual worker *vs.* farmer/housewives =0.56; 95% CI: 0.34–0.90); marital status (PR for never married *vs.* married = 1.35; 95% CI: 1.07–1.71); and nulliparity (PR for 0 *vs.* ≥3= 1.25; 95% CI: 1.05–1.49. Lifetime number of sexual partners, age at first sexual intercourse, and *care*HPV positivity were unrelated to screening history (Table 1).

Overall, 265 women (10%; 95% CI 9%–11%) were *care*HPV-positive with a significant variation by BHU (range: 5%–19%, I^2 = 63%, P-value<0.001) (Figure 4B). Table 2 shows the relationship between *care*HPV positivity and various women's characteristics. Significant risk factors for HPV positivity included region of Bhutan (PR for West *vs.* East =0·55; 95% CI: 0.30–1.00), marital status (PR for widow, separated or divorced *vs.* married=1·71; 95% CI: 1.21–2.41), number of pregnancies (PR for 1–2 *vs.* ≥3 =1.49; 95% CI: 1.15–1.93), and lifetime number of sexual partners (PR for ≥3 *vs.* 0–1=1.55; 95% CI: 1.05–2.27). *care*HPV positivity was not significantly associated with age, ethnicity, educational level, occupation, age at first sexual intercourse, and lack of previous screening (Table 2).

DISCUSSION

The findings from "REACH-Bhutan" show that community-based cervical cancer screening using self-collected samples and *care*HPV test is feasible in BHUs and can achieve high-coverage in rural Bhutan.

Seventy percent of women from rural communities responded favourably to the invitation to come to their BHU to undergo HPV screening. In comparison, in Thimphu, the Bhutanese capital, only 33% (95% CI: 29–37) of invited women of the same age group accepted to participate in a similar study.[5] However, participation significantly decreased with increasing age and travelling time between the village of residence and the BHU. The negative effect of living far away from a BHU was especially strong among older women. Furthermore, older age and large distance from BHUs were confirmed to be risk factors for lack of previous screening among the women who participated in our present study (Table 1).

The large majority of study participants reported to be illiterate farmers or housewives, currently married and sexually active. One-fifth reported two sexual partners or more, and 74% three children or more. Among study participants, 50% had never been screened before, confirming that rural areas are underserved compared to Thimphu, where 33% (95% CI: 30–36) of 30–60 year-old study participants had never been screened.[5] Despite the remarkable socio-demographic homogeneity of the rural communities included, participation in "REACH-Bhutan" and history of previous screening among participating women were significantly different among BHUs pointing to different levels of success in application of the national guidelines.

The varying degree of participation in our current study and of previous screening across BHUs may be also related to differences in ethnic composition. Indeed, lack of previous screening was significantly more frequent among women who belonged to the Lhotsampa (mostly Hindus) and Ngalop (mostly Buddhist) ethnic groups. It is, however, unclear whether Lhotsampa and Ngalop were more reluctant to be screened or lived in areas where BHUs were less committed to screening. For example, in the large BHU of Bara, at the western border with India, there was a suggestion that a historical absence of female HWs had had a negative impact on cervical screening attendance. The few literate women and those who were shopkeepers or manual workers reported more screening attendance.

Ten percent of women were *care*HPV-positive, i.e. a percentage only slightly lower than that found in Thimphu in the same age group, i.e. 14.1% (95% CI: 12.0–16.4).[12] Lifetime number of sexual partners, being widowed, separated or divorced, and having had 1 or 2 children (as compared to 3) were significantly associated with HPV positivity as reported in previous IARC HPV surveys.[13,14] Women in West Bhutan were less likely to be HPV positive than their counterparts in the east of the country and this finding is likely to reflect differences in lifestyle

sexual habits across rural communities or ethnic groups. In fact, the percentage of women who reported two or more sexual partners was 15% in the West versus 26% in the East.

The main strengths of the present study are the inclusion of a large number of women from many rural villages and the relatively high and accurately estimated participation of invited 30–60 year-old women. Study implementation was also characterised by logistic and technological challenges. We developed an mHealth platform and relied on the local mobile data network to ensure a timely and effective coordination of BHU HWs and mobile study teams across Bhutan, and to manage data collection, transmission, and real-time quality controls.[15,16] Furthermore, virtually all study participants had access to at least one mobile phone, greatly simplifying their recall for follow-up visits. The proposed diagnostic and treatment solutions also presented specific challenges. For example, while our study demonstrated the excellent acceptability and feasibility of organized screening based on self-collection, the *care*HPV platform turned out to be less reliable than expected and, even after completion of the initial training period, there still continued to be substantial wastage due to invalid *care*HPV runs. In addition, the original plan of rapidly recalling women and offering them triage and, if necessary, cryotherapy in one additional visit was not possible due to the hardship of roads and the frequent malfunctioning of cryotherapy in Bhutan.

A three-visit follow-up protocol is still ongoing and will be reported upon in a future publication. At end December 2016, 248 (94%) *care*HPV positive women had had a follow-up visit and 208 had undergone either cryotherapy (n=85) or loop electrosurgical excision procedure (LEEP, n=123). However, due to the heavy workload in the Pathology Department, the follow-up of women potentially in need of additional treatment is still incomplete.

CONCLUSION

The "REACH-Bhutan" study shows both the deep engagement of the Ministry of Health and HWs, and the willingness and resilience of Bhutanese women to comply with cervical cancer screening recommendations. It also highlights, however, the need to find new solutions to specific challenges, such as bringing self-collection closer to women, especially older ones living in the remotest areas, possibly in coordination with the decentralized offer of other primary health care activities, e.g., child vaccination, which is regularly brought from BHU to villages. Also, we underscore the need to assess the reliability of technological alternatives to the *care*HPV platform,[17] and to cryotherapy and LEEP,[18] for the treatment of precancerous lesions in countries such as Bhutan.[19]

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Contributors

IB, ST, SF, GC, and UT conceived and designed the study. IB, SF, GC, and UT drafted the manuscript. ST, TC, FL, VT, and MP critically revised the manuscript. All authors substantially contributed to the acquisition, analysis, and interpretation of data and approved the final manuscript.

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Disclaimer

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Competing interests

None declared.

Ethics approval

The present study had the approval of both the Research Ethical Board of the Bhutan Ministry of Health and the IARC Ethics Committee.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data sharing statement

No additional data are available.

References

1. Ervik M, Lam F, Ferlay J, Mery L, Soerjomataram I, Bray F. Cancer Today. 2016. http://gco.iarc.fr/today [accessed 11 November 2016].

2. Dhendup T, Tshering P. Cervical cancer knowledge and screening behaviors among female university graduates of year 2012 attending national graduate orientation program, Bhutan. *BMC Womens Health* 2014; **14**(1): 44.

3. Dorji T, Tshomo U, Phuntsho S, et al. Introduction of a National HPV vaccination program into Bhutan. *Vaccine* 2015; **33**(31): 3726-30.

4. Ministry of Health. National Health Survey. Thimphu, Bhutan: Ministry of Health 2012.

5. Baussano I, Tshomo U, Clifford GM, Tenet V, Tshokey T, Franceschi S. Cervical cancer screening program in Thimphu, Bhutan: population coverage and characteristics associated with screening attendance. *Bmc Womens Health* 2014; **14**: 147.

6. The World Bank. Rural population (% of total population). http://data.worldbank.org/indicator/SP.RUR.TOTL.ZS?locations=BT [accessed 11 January 2017].

7. Arbyn M, Anttila A, Jordan J, et al. European Guidelines for Quality Assurance in Cervical Cancer Screening. Second edition--summary document. *Ann Oncol* 2010; **21**(3): 448-58.

8. Ronco G, Dillner J, Elfstrom KM, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. *Lancet* 2014; **383**(9916): 524-32.

9. Arrossi S, Thouyaret L, Herrero R, et al. Effect of self-collection of HPV DNA offered by community health workers at home visits on uptake of screening for cervical cancer (the EMA study): a population-based cluster-randomised trial. *Lancet Glob Health* 2015; **3**(2): e85-94.

10. Tsu V, Jeronimo J. Saving the World's Women from Cervical Cancer. *N Engl J Med* 2016; **374**(26): 2509-11.

11. Qiao YL, Sellors JW, Eder PS, et al. A new HPV-DNA test for cervical-cancer screening in developing regions: a cross-sectional study of clinical accuracy in rural China. *Lancet Oncol* 2008; **9**(10): 929-36.

12. Tshomo U, Franceschi S, Dorji D, et al. Human papillomavirus infection in Bhutan at the moment of implementation of a national HPV vaccination programme. *BMC Infect Dis* 2014; **14**: 408.

13. Vaccarella S, Franceschi S, Herrero R, et al. Sexual behavior, condom use, and human papillomavirus: pooled analysis of the IARC human papillomavirus prevalence surveys. *Cancer Epidemiol Biomarkers Prev* 2006; **15**(2): 326-33.

14. Vaccarella S, Herrero R, Dai M, et al. Reproductive factors, oral contraceptive use, and human papillomavirus infection: pooled analysis of the IARC HPV prevalence surveys. *Cancer Epidemiol Biomarkers Prev* 2006; **15**(11): 2148-53.

15. Hall CS, Fottrell E, Wilkinson S, Byass P. Assessing the impact of mHealth interventions in low- and middle-income countries--what has been shown to work? *Glob Health Action* 2014; **7**: 25606.

16. Howitt P, Darzi A, Yang GZ, et al. Technologies for global health. *Lancet* 2012; **380**(9840): 507-35.

17. Cubie HA, Morton D, Kawonga E, et al. HPV prevalence in women attending cervical screening in rural Malawi using the cartridge-based Xpert(R) HPV assay. *J Clin Virol* 2016; **87**: 1-4.

18. Dolman L, Sauvaget C, Muwonge R, Sankaranarayanan R. Meta-analysis of the efficacy of cold coagulation as a treatment method for cervical intraepithelial neoplasia: a systematic review. *BJOG* 2014; **121**(8): 929-42.

19. Viviano M, Kenfack B, Catarino R, et al. Feasibility of thermocoagulation in a screen-and-treat approach for the treatment of cervical precancerous lesions in sub-Saharan Africa. *Bmc Womens Health* 2017; **17**(1): 2.

Table 1: Prevalence ratios (PR) for lack of previous cervical cancer screening and corresponding 95% confidence intervals (CI) according to selected characteristics, Bhutan, 2016

Characteristic	N tested	Never screened N (%)	Adjusted PR*	95% CI
Age (years)				
30–39	1139	497 (43.6)	1	–
40–49	818	407 (49.8)	1.14	1.05–1.24
≥50	633	383 (60.5)	1.36	1.26–1.47
χ^2_1 *for trend*			*p<0.001*	
Region				
East	1500	625 (41.7)	1	–
West	1090	662 (60.7)	2.84	2.05–3.95
Travel time from village to BHU (hours)†				
<1	656	285 (43.5)	1	–
1–5	1287	576 (44.8)	1.01	0.91–1.12
Missing	6	4 (66.7)	–	–
≥6–	641	422 (65.8)	1.53	1.38–1.69
χ^2_1 *for trend*			*p<0.001*	
Ethnicity†				
Scharchop	1435	615 (42.9)	1	–
Ngalop	340	236 (69.4)	1.65	1.52–1.80
Lhotsampa	686	401 (58.5)	1.41	1.29–1.53
Khengpa	113	26 (23.0)	0.58	0.41–0.81
Other	16	9 (56.3)	1.49	0.97–2.31
Education level				
Illiterate	2372	1191 (50.2)	1	–
Literate	218	96 (44.0)	0.85	0.73–0.99
Current occupation				
Farmer/ housewife	2488	1254 (50.4)	1	–
Shopkeeper/saleswoman/manual worker	53	12 (22.6)	0.56	0.34–0.90
Clerical staff/teacher/health worker/nun	49	21 (42.9)	0.97	0.72–1.31
Marital status†				
Married/living as married	2390	1175 (49.2)	1	–

Never married	21	16 (76.2)	1.35	1.07–1.71
Widow/separated/divorced	179	96 (53.6)	1.05	0.92–1.21
Number of pregnancies†				
0	67	43 (64.2)	1.25	1.05–1.49
1–2	604	285 (47.2)	1.03	0.94–1.14
≥3	1919	959 (50.0)	1	–
χ_1^2 *for trend*			*p=0.057*	
Lifetime number of sexual partners				
0–1	2020	983 (48.7)	1	–
2	341	166 (48.7)	0.97	0.88–1.08
≥3	214	126 (58.9)	0.98	0.88–1.09
Prefer not to answer	15	12 (80.0)	–	–
χ_1^2 *for trend*			*p=0.601*	
Age at first sexual intercourse (years)‡				
9–14	245	124 (50.6)	1	–
15–16	515	235 (45.6)	0.93	0.81–1.06
17–19	968	452 (46.7)	1.00	0.89–1.12
≥20	707	365 (51.6)	1.02	0.91–1.15
Prefer not to answer / unknown	143	101 (70.6)	–	–
χ_1^2 *for trend*			*p=0.151*	
HPV infection				
Negative	2325	1168 (50.2)	1	–
Positive	265	119 (44.9)	1.01	0.91–1.13

CI=confidence interval; HPV=human papillomavirus; PR=prevalence ratio.*Adjusted for age (3 classes: 30–39; 40–49; 50+) and Basic Health Unit as appropriate. †Adjusted for age only – when adjusted for BHU, model does not converge. ‡Among sexually active women.

Table 2: Prevalence ratios (PR) for high-risk human papillomavirus (HPV) positivity and corresponding 95% confidence intervals (CI) according to selected characteristics, Bhutan, 2016

Characteristic	N tested	*care*-HPV positive N (%)	Adjusted PR*	95% CI
Age (years)				
30–39	1139	129 (11.3)	1	–
40–49	818	76 (9.3)	0.80	0.62–1.05
≥50	633	60 (9.5)	0.79	0.59–1.05
χ^2_1 *for trend*			*p=0.073*	
Region				
East	1500	173 (11.5)	1	–
West	1090	92 (8.4)	0.55	0.30–1.00
Ethnicity				
Scharchop	1435	164 (11.4)	1	–
Ngalop	340	28 (8.2)	0.68	0.31–1.49
Lhotsampa	686	58 (8.5)	0.67	0.33–1.38
Khengpa	113	15 (13.3)	1.80	0.31–10.5
Other	16	0 (0)	–	–
Education level				
Illiterate	2372	238 (10.0)	1	–
Literate	218	27 (12.4)	1.33	0.90–1.95
Current occupation				
Farmer/ housewife	2488	253 (10.2)	1	–
Shopkeeper/saleswoman/manual worker	53	3 (5.7)	0.56	0.18–1.68
Clerical staff/teacher/health worker/nun	49	9 (18.4)	1.65	0.90–3.01
Marital status				
Married/living as married	2390	232 (9.7)	1	–
Never married	21	3 (14.3)	1.53	0.54–4.38
Widow/separated/divorced	179	30 (16.8)	1.71	1.21–2.41
Number of pregnancies				
0	67	8 (11.9)	1.29	0.66–2.49
1–2	604	83 (13.7)	1.49	1.15–1.93

≥3	1919	174 (9.1)	1	–
χ_1^2 *for trend*			*p=0.007*	
Lifetime number of sexual partners				
0–1	2020	198 (9.8)	1	–
2	341	36 (10.6)	1.20	0.85–1.69
≥3	214	31 (14.5)	1.55	1.05–2.27
Prefer not to answer	15	0 (0.0)	–	–
χ_1^2 *for trend*			*p=0.022*	
Age at first sexual intercourse (years)†				
9–14	245	21 (8.6)	1	–
15–16	515	48 (9.3)	1.05	0.64–1.71
17–19	968	109 (11.3)	1.20	0.76–1.89
≥20	707	72 (10.2)	1.11	0.69–1.79
Prefer not to answer / unknown	143	14 (9.8)	–	–
χ_1^2 *for trend*			*p=0.576*	
History of PAP smear (years)				
Ever	1303	146 (11.2)	1	–
Never	1287	119 (9.3)	1.06	0.81–1.39

CI=confidence interval; HPV=human papillomavirus; PR=prevalence ratio.*Adjusted for age (3 classes: 30–39; 40–49; 50+) and Basic Health Unit as appropriate. †Among sexually active women.

Figure Legends

Figure 1: Map of Bhutan with study sites and predominant ethnic groups.*

The size of each dot is proportional to the size of the target population of each centre.

Figure 2: Participation (%) and corresponding 95% confidence intervals by Basic Health Unit, region, and age group, Bhutan, 2016.

Figure 3: Effect of travel time (on foot) on participation in REACH Bhutan, by age group, Bhutan, 2016.

Figure 4: Percent of A) lack of previous cervical cancer screening; and B) *care*-HPV positivity by Basic Health Unit and overall in rural areas and in Thimphu, Bhutan, 2016.

*women aged 30-60 years in Tshomo et al 2014.

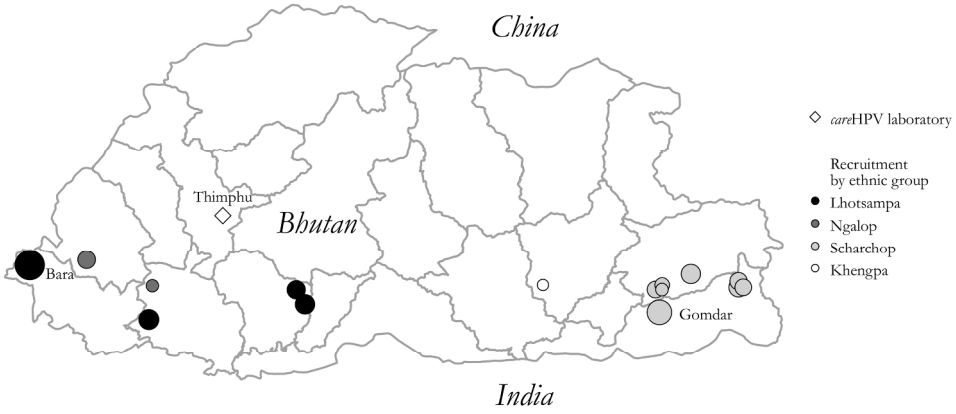


Figure 1: Map of Bhutan with study sites and predominant ethnic groups.*
The size of each dot is proportional to the size of the target population of each centre.

233x165mm (300 x 300 DPI)

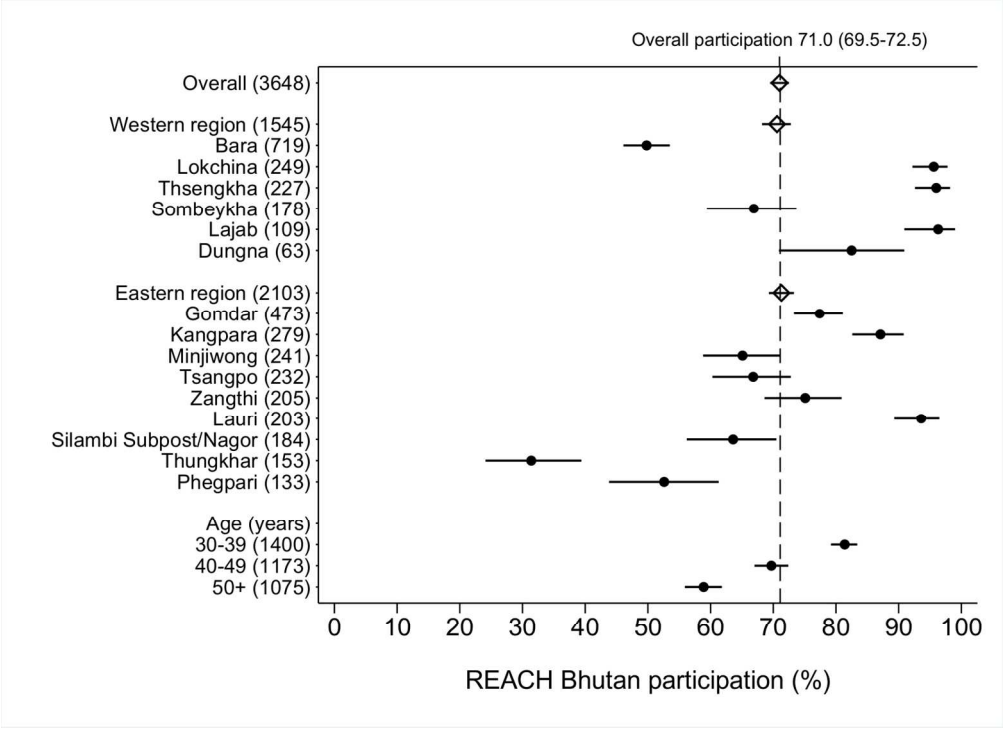


Figure 2: Participation (%) and corresponding 95% confidence intervals by Basic Health Unit, region, and age group, Bhutan, 2016.

139x101mm (300 x 300 DPI)

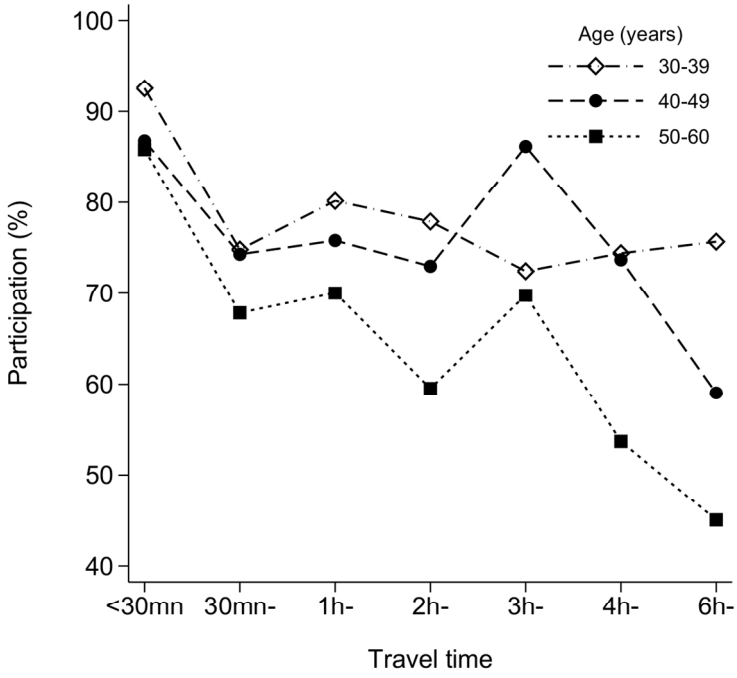


Figure 3: Effect of travel time (on foot) on participation in REACH Bhutan, by age group, Bhutan, 2016.

139x101mm (300 x 300 DPI)

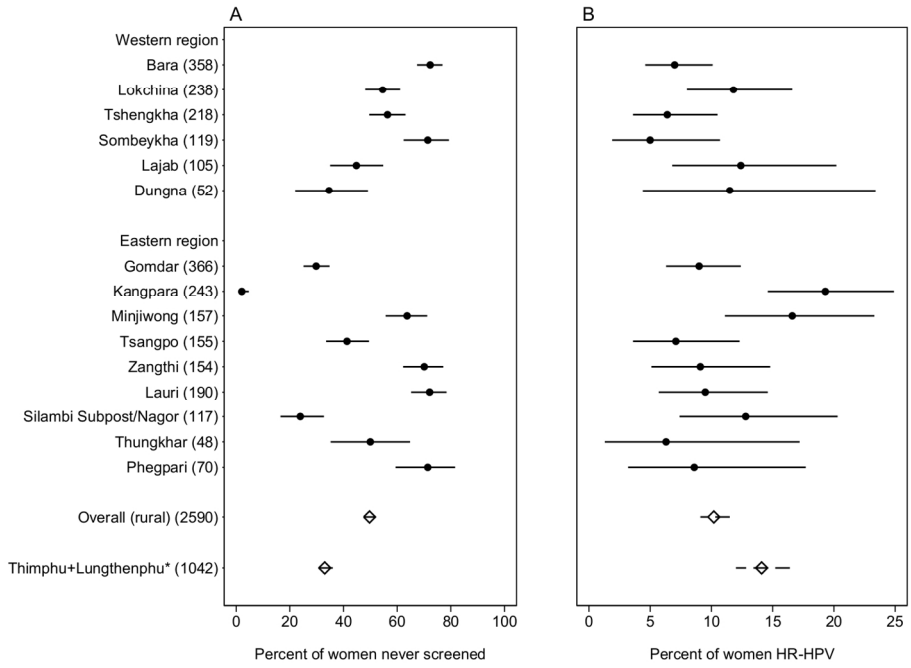
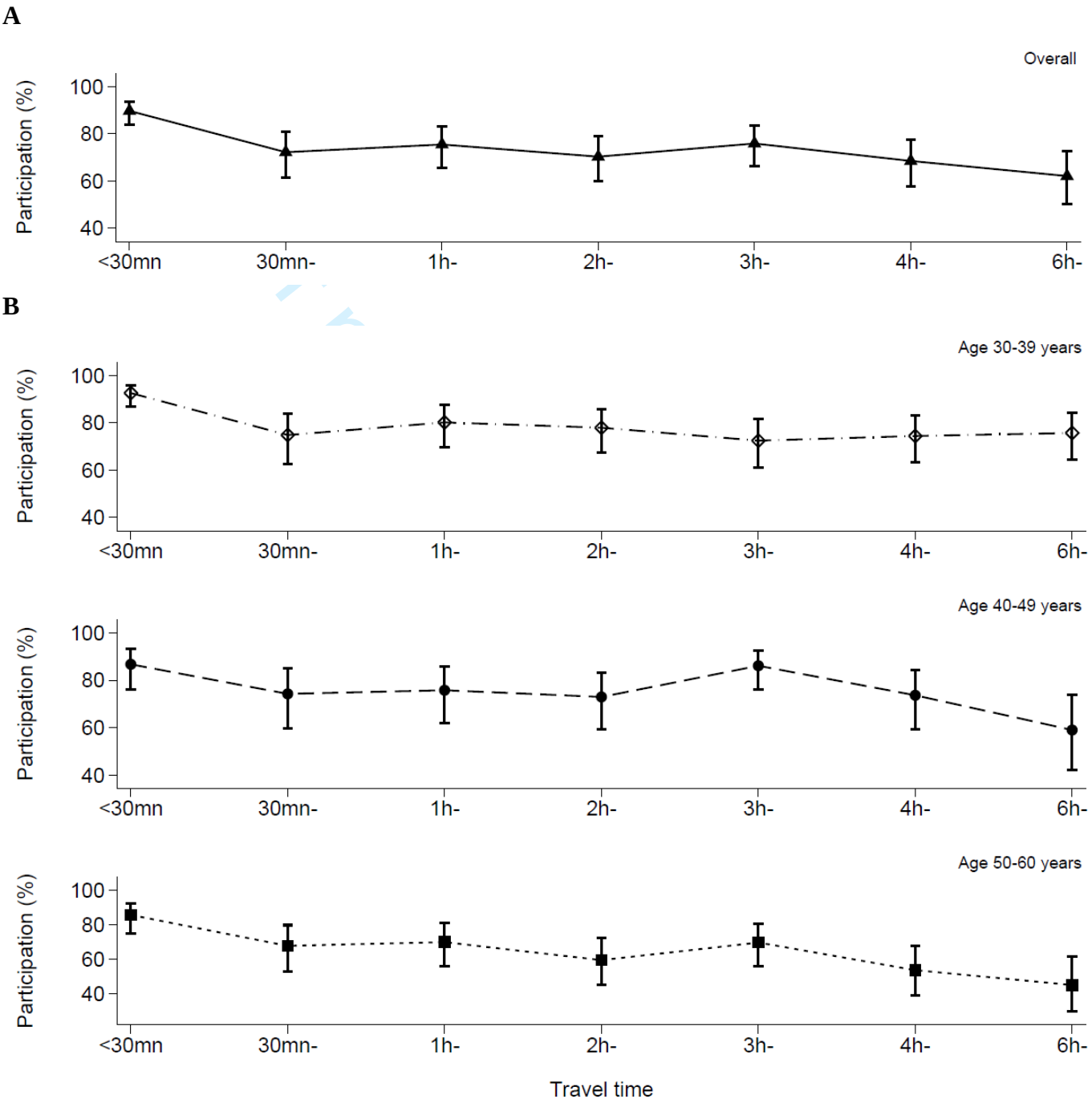


Figure 4: Percent of A) lack of previous cervical cancer screening; and B) care-HPV positivity by Basic Health Unit and overall in rural areas and in Thimphu, Bhutan, 2016.*women aged 30-60 years in Tshomo et al 2014.

139x101mm (300 x 300 DPI)

Appendix Figure 1: Effect of travel time on participation (%) in REACH Bhutan, A) overall, B) by age group with 95% confidence interval, Bhutan, 2016



STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1/3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	7
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	Fig 2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9; tables 1 & 2
		(b) Indicate number of participants with missing data for each variable of interest	9, tables 1 & 2
Outcome data	15*	Report numbers of outcome events or summary measures	9, Figure 2,4 and Tables
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted	9

		estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Tables 1 & 2
		(*b*) Report category boundaries when continuous variables were categorized	Tables 1 & 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Figure 3
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8, Figure 3
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Cervical cancer screening in rural Bhutan with the careHPV test on self-collected samples: an ongoing cross-sectional, population-based study (REACH-Bhutan)

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Secondary Subject Heading:	Public health
Keywords:	Cervical cancer screening, self-collection, careHPV, rural population, Bhutan

Title: Cervical cancer screening in rural Bhutan with the *care*HPV test on self-collected samples: an ongoing cross-sectional, population-based study (REACH-Bhutan)

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Brief title: Cervical cancer screening in rural Bhutan

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Main text word count: approx. 2565 (limit 4000 words).

Abstract word count: 300 words

Number of references: 24.

Tables: 2; Figures: 4

Appendix: 1 including 1 figure

Abstract (words=300)

Objectives. The Bhutanese Screening Programme recommends a Pap smear every three years for women aged 25–65 years, and coverage ranges from 20% to 60%, being especially challenging in rural settings. The "REACH-Bhutan" study was conducted to assess the feasibility and outcomes of a novel approach to cervical cancer screening in rural Bhutan.

Design. Cross-sectional, population-based study of cervical cancer screening based on the *care*HPV test on self-collected samples.

Setting. Women were recruited in rural primary health care centres, i.e. Basic Health Units (BHU), across Bhutan.

Participants. Overall, 3,648 women aged 30–60 were invited from 15 BHUs differing in accessibility, size, and ethnic composition of the population.

Interventions. Participants provided a self-collected cervico-vaginal sample and were interviewed. Samples were tested using *care*HPV in Thimphu (the Bhutanese capital) referral laboratory.

Main outcome measures. Screening participation by geographic area, centre, age, and travelling time. Previous screening history and *care*HPV-positivity by selected characteristics of the participants.

Results. In April/May 2016, 2590 women (median age: 41) were enrolled. Study participation was 71% and significantly heterogeneous by BHU (range: 31%–96%). Participation decreased with increase in age (81% in 30–39 year-old women; 59% in ≥50 years), and travelling time (90% in women living <30 minutes from the BHU *versus* 62% among those >6 hours away). 50% participants reported no previous screening, with the proportion of never-screened women varying significantly by BHU (range: 2%–72%). 265 women (10%; 95%CI 9%–11%) were

*care*HPV-positive, with a significant variation by BHU (range: 5%–19%) and number of sexual partners (prevalence ratio for ≥3 *vs.* 0–1=1.55; 95% CI: 1.05–2.27).

Conclusions:

Community-based cervical cancer screening by testing self-collected samples for HPV can achieve high coverage in rural Bhutan. However, solutions to bring self-collection, HPV testing, and precancer treatment even closer to the remotest villages are needed.

Keywords: Cervical cancer screening; self-collection; *care*HPV; rural population; Bhutan.

Article summary section:

Strengths and limitations of this study

- The study was conducted countrywide in a range of rural primary health care centres, which varied in accessibility, size, and ethnic composition of the target population.
- The target population of each centre was enumerated on the basis of up-to-date and detailed demographic surveys.
- A reliable local mobile data network ensured timely and effective study coordination, data collection, and quality controls.
- The proposed diagnostic and treatment solutions presented specific challenges and were less reliable than expected.

INTRODUCTION

Cervical cancer represents the most common cancer among females in Bhutan, with an age-standardised incidence rate of approximately 13 cases per 100,000 person years.[1] The country is strongly engaged in the prevention of cervical cancer. In the year 2000, the Ministry of Health launched a national cytology-based screening programme, and a Pap smear is currently recommended every three years to women aged 25–65 years.[2] In the year 2010, Bhutan was the first low/middle-income country (LMIC) to initiate a successful national vaccination programme against human papillomavirus (HPV) with >90% coverage in girls age 12–18 years.[3] Conversely, screening coverage is much lower and fairly heterogeneous across the country, ranging from 20% to 60% of the target population in different provinces.[4 5] In rural areas, where approximately 60% of the Bhutanese live,[6] cytology-based screening campaigns are occasionally conducted but coverage remains poor,[4] and follow-up and treatment of screening-positive women is challenging.

Organized screening programmes are demanding in terms of administrative, human, financial, and logistic resources. Pap smear-based programmes require frequent visits, trained staff, and strict quality control. In contrast, HPV-based screening allows for longer screening intervals,[7 8] self-sampling,[9] and automation of the diagnostic procedure. It is therefore an attractive option to improve the acceptability and cost-effectiveness of screening.[10]

In 2016, the Ministry of Health of Bhutan and the International Agency for Research on Cancer (IARC) implemented the "REACH-Bhutan" study to assess the feasibility, outcomes, and challenges of cervical cancer screening based on the *care*HPV test on self-collected samples among women 30–60 years of age in rural areas of the country. In the current report, we describe the study design, target population, recruitment and sample collection methods, key

characteristics of study participants, and patterns of participation. Details on the performance of *care*HPV testing and clinical management of *care*HPV-positive women will be reported in future publications.

MATERIAL AND METHODS

Study population and recruitment

Our study targeted women aged 30–60 years living in rural areas; recruitment and sample collection took place from April to May 2016 in Basic Health Units (BHUs), the facilities that provide primary health care to the Bhutanese population. The Bhutanese principal investigator (UT) selected 15 BHUs that differed by accessibility, size, and ethnic composition of the served population (Figure 1). Lists of household members in each village were obtained from the BHU survey produced yearly for demographic purposes.

Local health workers (HWs) familiar with community mobilization were instructed by the principal investigator in the specifics of self-sampling for cervical screening, and visited the villages served by the selected BHUs to invite eligible women to come to the BHU to participate in the study on specified dates. The benefits of cervical cancer screening and the purposes of the study were explained to the women during public invitation sessions. All women from a given village were invited to attend screening at the BHUs on the same date. Pregnant women, women with mental disability, who had undergone hysterectomy or planned to leave the study area in the next 6 months were not eligible.

On the appointment date, two or three HWs and one of the two mobile study teams (one for East and one for West Bhutan, each composed of two nurses and supervised by a gynaecologist) provided additional information on the study, collected an informed consent

form, and administered a short electronic questionnaire on the BHU's premises. The entire process required less than 20 minutes and certain BHUs were able to manage more than 100 women per day. Whenever possible, the recently introduced national citizenship identification number (CID), which uniquely identifies Bhutanese citizens, and at least one mobile telephone number were recorded for follow-up purposes.

Sample collection, transportation, and laboratory analysis

Each participant was asked to provide a self-collected cervico-vaginal sample using a *care*Brush. They were instructed to insert the brush deep into the vagina and rotate it 3 to 5 times before dipping it in a tube containing *care*HPV collection medium. The study team nurse provided guidance to the participants on how to perform self-collection but did not attend the self-collection procedure. Tubes were then stored in fridges until they were transported in cool boxes to the central laboratory of Jigme Dorji Wangchuck National Referral Hospital (JDWNRH) in Thimphu. Samples were stored at ~4º C and an aliquot was tested using the *care*HPV platform (Qiagen Corporation, Gaithersburg, MD, USA) according to the manufacturer's instructions. The *care*HPV test is a validated signal-amplification, rapid batch diagnostic test for the detection of DNA of 13 high risk (HR) HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and HPV66.[11] *care*HPV results were communicated by the central laboratory as soon as possible to each BHU (after a median of 11 days; inter-quartile range: 9 to 33 days) to arrange follow-up of *care*HPV-positive women and of a random fraction of *care*HPV-negative women, as a quality control measure.

Data collection and statistical analyses

All information on study participants and biological samples were collected and stored on portable electronic devices. Whenever a mobile data network was accessible, the stored data were uploaded to the IARC server and portable devices were synchronized with the most recent version of the study database. The distribution of the target population in each BHU by age group and by village of residence was obtained from the demographic survey conducted in year 2015. Travelling time was estimated from each village of residence to the corresponding BHU. On account of the socio-geographic characteristics of the study areas, most women had to reach the BHU on foot. We subdivided the travelling time into seven categories (i.e. <30 minutes, 30 to 59 minutes, 1 hour to 1 hour 59 minutes, 2 hours to 2 hours 59 minutes, 3 hours to 3 hours 59 minutes, 4 hours to 5 hours 59 minutes, and 6 or more hours), and fitted a mixed-effect logistic model to data to assess the influence of travelling time on study participation, with BHUs as clusters.

Among participating women, we assessed prevalence ratios (PR) and corresponding 95% confidence intervals (CI) for lack of previous cervical cancer screening and *care*HPV positivity according to selected characteristics using binomial regression models with a log link and adjustment for age group (30–39, 40–49, and ≥50 years) and BHU of recruitment, as appropriate. For each variable of interest, missing or undisclosed (i.e. labelled as "prefer not to answer") information were treated as not informative and excluded from the analyses.

RESULTS

Out of 15 BHUs included in "REACH-Bhutan", 6 are located in West Bhutan and 9 in East Bhutan (Figure 1). They serve a total of 3,648 women age 30–60 years (Figure 2). Overall participation was 71% (95% CI: 69.5%–72.5%) and was similar in the West and the East. It

was, however, significantly heterogeneous by BHU (range: 31%–96%, I-square 99%, p<0.001), and age group (81% in 30–39 year-old women but only 59% in ≥50 years). Participation steadily decreased with the increase in travelling time from village to BHU being 90% (95% CI: 84%–94%) for women living less than 30 minutes from the BHU but 62% (95% CI: 50%–73%) among those 6 hours away or more (see appendix p1). The influence of travelling time strongly increased with a woman's age: the drop in participation between 30 minutes and 6 hours was from 93% (95% CI: 87%–96%) to 76% (95% CI: 64%–84%) among women 30–39 years old, but from 86% (95% CI: 75%–92%) to 45% (95% CI: 30%–62%) among women ≥50 years (Figure 3). The village of residence was not reported by 6 women or did not belong to the BHU catchment area (7 women). These women did not contribute to the analysis reported in Figure 3.

A total 2,590 women accepted the invitation and came to a BHU (median age: 41 years; inter-quartile range: 35 to 49 years). Ninety-nine percent belonged to 4 ethnic groups: Scharchop (55%); Lhotsampa (27%); Ngalop (13%); and Khengpa (4%), each group predominating in at least one BHU (Figure 1). All but two women provided a telephone number and 60% provided their identity card number. Most women stated that self-sampling was easy to perform (96%) and painless (90%). Macroscopic blood traces were observed in 3·5% of sample of non-menstruating women (data not shown).

Fifty percent of participants reported having had previous screening (Figure 4A) and, among them, 83% had had a Pap smear in the last 4 years (data not shown). The proportion of never-screened women varied across individual BHUs (range: 2%–72%, I2 = 99%, P-value<0.001). Table 1 shows the characteristics that were significantly associated with lack of screening, i.e., age group (PR for ≥50 *vs.* 30–39 years =1.36; 95% CI: 1.26–1.47); region (PR for West *vs.* East=2·84; 95% CI: 2.05–3.95); travelling time from village to BHU (PR for ≥6 hours *vs.* <1

hour =1.53; 95% CI: 1.38–1.69); ethnicity (PR *vs.* Scharchop = 1.65; 95% CI: 1.52–1.80 for Ngalop; 1.41; 95% CI: 1.29–1.53 for Lhotsampa; and 0.58; 95% CI: 0.41–0.81 for Khengpa); educational level (PR for literate *vs.* illiterate =0.85; 95% CI: 0.73–0.99); occupation (PR for shopkeepers/saleswoman/manual worker *vs.* farmer/housewives =0.56; 95% CI: 0.34–0.90); marital status (PR for never married *vs.* married = 1.35; 95% CI: 1.07–1.71); and nulliparity (PR for 0 *vs.* ≥3= 1.25; 95% CI: 1.05–1.49. Lifetime number of sexual partners, age at first sexual intercourse, and *care*HPV positivity were unrelated to screening history (Table 1).

Overall, 265 women (10%; 95% CI 9%–11%) were *care*HPV-positive with a significant variation by BHU (range: 5%–19%, I^2 = 63%, P-value<0.001) (Figure 4B). Table 2 shows the relationship between *care*HPV positivity and various women's characteristics. Significant risk factors for HPV positivity included region of Bhutan (PR for West *vs.* East =0·55; 95% CI: 0.30–1.00), marital status (PR for widow, separated or divorced *vs.* married=1·71; 95% CI: 1.21–2.41), number of pregnancies (PR for 1–2 *vs.* ≥3 =1.49; 95% CI: 1.15–1.93), and lifetime number of sexual partners (PR for ≥3 *vs.* 0–1=1.55; 95% CI: 1.05–2.27). careHPV positivity was not significantly associated with age, ethnicity, educational level, occupation, age at first sexual intercourse, and lack of previous screening (Table 2).

DISCUSSION

The findings from "REACH-Bhutan" show that community-based cervical cancer screening using self-collected samples and *care*HPV test is feasible in BHUs and can achieve high-coverage in rural Bhutan. Our study, therefore, can be added to an increasing number of evaluations of the implementation of *care*-HPV screening in under-served populations of Asia,[12-14] Africa,[15-17] and Latin America.[18 19]

Seventy percent of women from rural communities responded favourably to the invitation to come to their BHU to undergo HPV screening. In comparison, in Thimphu, the Bhutanese capital, only 33% (95% CI: 29–37) of invited women of the same age group accepted to take part in a study of clinician collected samples.[5] However, participation significantly decreased with increasing age and travelling time between the village of residence and the BHU. The negative effect of living far away from a BHU was especially strong among older women. Furthermore, older age and large distance from BHUs were confirmed to be risk factors for lack of previous screening among the women who participated in our present study (Table 1).

The large majority of study participants reported to be illiterate farmers or housewives, currently married and sexually active. One-fifth reported two sexual partners or more, and 74% three children or more. Among study participants, 50% had never been screened before, confirming that rural areas are underserved compared to Thimphu, where 33% (95% CI: 30–36) of 30–60 year-old study participants had never been screened.[5] Despite the remarkable socio-demographic homogeneity of the rural communities included, participation in "REACH-Bhutan" and history of previous screening among participating women were significantly different among BHUs pointing to different levels of success in application of the national guidelines.

The varying degree of participation in our current study and of previous screening across BHUs may be also related to differences in ethnic composition. Indeed, lack of previous screening was significantly more frequent among women who belonged to the Lhotsampa (mostly Hindus) and Ngalop (mostly Buddhist) ethnic groups. It is, however, unclear whether Lhotsampa and Ngalop were more reluctant to be screened or lived in areas where BHUs were less committed to screening. For example, in the large BHU of Bara, at the western border with India, there was a suggestion that a historical absence of female HWs had had a negative impact

on cervical screening attendance. The few literate women and those who were shopkeepers or manual workers reported more screening attendance.

Ten percent of women were *care*HPV-positive, i.e. a percentage only slightly lower than that found in Thimphu in the same age group, i.e. 14.1% (95% CI: 12.0–16.4).[20] Lifetime number of sexual partners, being widowed, separated or divorced, and having had 1 or 2 children (as compared to 3) were significantly associated with HPV positivity as reported in previous IARC HPV surveys.[21 22] Women in West Bhutan were less likely to be HPV positive than their counterparts in the east of the country and this finding is likely to reflect differences in lifestyle sexual habits across rural communities or ethnic groups. In fact, the percentage of women who reported two or more sexual partners was 15% in the West versus 26% in the East.

The main strengths of the present study are the inclusion of a large number of women from many rural villages and the relatively high and accurately estimated participation of invited 30–60 year-old women. We developed an mHealth platform and relied on the local mobile data network to ensure a timely and effective coordination of BHU HWs and mobile study teams across Bhutan, and to manage data collection, transmission, and real-time quality controls.[23 24] Furthermore, virtually all study participants had access to at least one mobile phone, greatly simplifying their recall for follow-up visits.

However, study implementation was also characterised by logistic and technological challenges. For example, although the central laboratory in Thimphu was able to deliver *care*HPV results to each BHU in a median of 11 days after screening, the *care*HPV platform turned out to be less reliable than expected and, even after completion of the initial training period, there still continued to be substantial wastage due to invalid *care*HPV runs. In addition, the original plan of rapidly recalling women and offering them colposcopy triage and, if

necessary, cryotherapy in each BHU was hindered by difficulties in transport and/or malfunction of cryotherapy equipment.

At end March 2017, 248 (94%) *care*HPV positive women had had a follow up visit and 217 had undergone either cryotherapy (n=88) or loop electrosurgical excision procedure (LEEP, n=129). Histological ascertainment of cervical specimens from these women (as well as from a subset of care-HPV negative women) is still ongoing, and clinical outcomes will be the subject of a future publication.

CONCLUSION

The "REACH-Bhutan" study shows both the readiness of the Bhutanese Health System, and the willingness and resilience of Bhutanese women, to comply with cervical cancer screening algorithms based on self-collection for HPV testing. It also highlights, however, the need to find new solutions to specific challenges, such as bringing self-collection even closer to women, especially older ones living in the remotest areas, possibly in coordination with the decentralized offer of other primary health care activities, e.g., child vaccination, which are regularly brought from BHUs to villages.

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Contributors

IB, ST, SF, GC, and UT conceived and designed the study. IB, SF, GC, and UT drafted the manuscript. ST, TC, FL, VT, and MP critically revised the manuscript. All authors substantially contributed to the acquisition, analysis, and interpretation of data and approved the final manuscript.

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Disclaimer

The funders had no role in study design, data collection and analysis, decision to publish or in the preparation of the manuscript.

Competing interests

None declared.

Ethics approval

The present study had the approval of both the Research Ethical Board of the Bhutan Ministry of Health and the IARC Ethics Committee.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data sharing statement

No additional data are available.

References

1. Ervik M, Lam F, Ferlay J, et al. Cancer Today Lyon, France: International Agency for Research on Cancer. 2016 [Available from: http://gco.iarc.fr/today accessed 11 November 2016].
2. Dhendup T, Tshering P. Cervical cancer knowledge and screening behaviors among female university graduates of year 2012 attending national graduate orientation program, Bhutan. *BMC Womens Health* 2014;14(1):44.
3. Dorji T, Tshomo U, Phuntsho S, et al. Introduction of a National HPV vaccination program into Bhutan. *Vaccine* 2015;33(31):3726-30. doi: 10.1016/j.vaccine.2015.05.078
4. Ministry of Health. National Health Survey. Thimphu, Bhutan: Ministry of Health 2012.
5. Baussano I, Tshomo U, Clifford GM, et al. Cervical cancer screening program in Thimphu, Bhutan: population coverage and characteristics associated with screening attendance. *Bmc Womens Health* 2014;14:147. doi: 10.1186/s12905-014-0147-0
6. The World Bank. Rural population (% of total population). [Available from: http://data.worldbank.org/indicator/SP.RUR.TOTL.ZS?locations=BT accessed 11 January 2017].
7. Arbyn M, Anttila A, Jordan J, et al. European Guidelines for Quality Assurance in Cervical Cancer Screening. Second edition--summary document. *Ann Oncol* 2010;21(3):448-58. doi: 10.1093/annonc/mdp471
8. Ronco G, Dillner J, Elfstrom KM, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. *Lancet* 2014;383(9916):524-32. doi: 10.1016/S0140-6736(13)62218-7

9. Arrossi S, Thouyaret L, Herrero R, et al. Effect of self-collection of HPV DNA offered by community health workers at home visits on uptake of screening for cervical cancer (the EMA study): a population-based cluster-randomised trial. *Lancet Glob Health* 2015;3(2):e85-94. doi: 10.1016/S2214-109X(14)70354-7

10. Tsu V, Jeronimo J. Saving the World's Women from Cervical Cancer. *N Engl J Med* 2016;374(26):2509-11. doi: 10.1056/NEJMp1604113

11. Qiao YL, Sellors JW, Eder PS, et al. A new HPV-DNA test for cervical-cancer screening in developing regions: a cross-sectional study of clinical accuracy in rural China. *Lancet Oncol* 2008;9(10):929-36. doi: 10.1016/S1470-2045(08)70210-9

12. Qiao YL, Sellors JW, Eder PS, et al. A new HPV-DNA test for cervical-cancer screening in developing regions: a cross-sectional study of clinical accuracy in rural China. *Lancet Oncol* 2008;9(10):929-36.

13. Tuerxun G, Yukesaier A, Lu L, et al. Evaluation of careHPV, Cervista Human Papillomavirus, and Hybrid Capture 2 Methods in Diagnosing Cervical Intraepithelial Neoplasia Grade 2+ in Xinjiang Uyghur Women. *Oncologist* 2016;21(7):825-31. doi: 10.1634/theoncologist.2015-0447 [published Online First: 2016/06/19]

14. Labani S, Asthana S. Age-specific performance of careHPV versus Papanicolaou and visual inspection of cervix with acetic acid testing in a primary cervical cancer screening. *J Epidemiol Community Health* 2016;70(1):72-7. doi: 10.1136/jech-2015-205851 [published Online First: 2015/08/08]

15. Obiri-Yeboah D, Adu-Sarkodie Y, Djigma F, et al. Options in human papillomavirus (HPV) detection for cervical cancer screening: comparison between full genotyping and a rapid

qualitative HPV-DNA assay in Ghana. *Gynecologic oncology research and practice* 2017;4:5. doi: 10.1186/s40661-017-0041-1 [published Online First: 2017/03/09]

16. Segondy M, Kelly H, Magooa MP, et al. Performance of careHPV for detecting high-grade cervical intraepithelial neoplasia among women living with HIV-1 in Burkina Faso and South Africa: HARP study. *Br J Cancer* 2016;115(4):425-30. doi: 10.1038/bjc.2016.207 [published Online First: 2016/07/20]
17. Bansil P, Lim J, Byamugisha J, et al. Performance of Cervical Cancer Screening Techniques in HIV-Infected Women in Uganda. *J Low Genit Tract Dis* 2015;19(3):215-9. doi: 10.1097/lgt.0000000000000090 [published Online First: 2015/01/01]
18. Lorenzi AT, Fregnani JH, Possati-Resende JC, et al. Can the careHPV test performed in mobile units replace cytology for screening in rural and remote areas? *Cancer Cytopathol* 2016;124(8):581-8. doi: 10.1002/cncy.21718 [published Online First: 2016/04/14]
19. Maza M, Alfaro K, Garai J, et al. Cervical cancer prevention in El Salvador (CAPE)-An HPV testing-based demonstration project: Changing the secondary prevention paradigm in a lower middle-income country. *Gynecologic oncology reports* 2017;20:58-61. doi: 10.1016/j.gore.2017.02.011 [published Online First: 2017/03/25]
20. Tshomo U, Franceschi S, Dorji D, et al. Human papillomavirus infection in Bhutan at the moment of implementation of a national HPV vaccination programme. *BMC Infect Dis* 2014;14:408. doi: 10.1186/1471-2334-14-408
21. Vaccarella S, Franceschi S, Herrero R, et al. Sexual behavior, condom use, and human papillomavirus: pooled analysis of the IARC human papillomavirus prevalence surveys. *Cancer Epidemiol Biomarkers Prev* 2006;15(2):326-33. doi: 10.1158/1055-9965.EPI-05-0577

22. Vaccarella S, Herrero R, Dai M, et al. Reproductive factors, oral contraceptive use, and human papillomavirus infection: pooled analysis of the IARC HPV prevalence surveys. *Cancer Epidemiol Biomarkers Prev* 2006;15(11):2148-53. doi: 10.1158/1055-9965.EPI-06-0556

23. Hall CS, Fottrell E, Wilkinson S, et al. Assessing the impact of mHealth interventions in low- and middle-income countries--what has been shown to work? *Glob Health Action* 2014;7:25606. doi: 10.3402/gha.v7.25606

24. Howitt P, Darzi A, Yang GZ, et al. Technologies for global health. *Lancet* 2012;380(9840):507-35. doi: 10.1016/S0140-6736(12)61127-1

Table 1: Prevalence ratios (PR) for lack of previous cervical cancer screening and corresponding 95% confidence intervals (CI) according to selected characteristics, Bhutan, 2016

Characteristic	N tested	Never screened N (%)	Adjusted PR*	95% CI
Age (years)				
30–39	1139	497 (43.6)	1	–
40–49	818	407 (49.8)	1.14	1.05–1.24
≥50	633	383 (60.5)	1.36	1.26–1.47
χ^2_1 *for trend*			*p<0.001*	
Region				
East	1500	625 (41.7)	1	–
West	1090	662 (60.7)	2.84	2.05–3.95
Travel time from village to BHU (hours)†				
<1	656	285 (43.5)	1	–
1–5	1287	576 (44.8)	1.01	0.91–1.12
Missing	6	4 (66.7)	–	–
≥6–	641	422 (65.8)	1.53	1.38–1.69
χ^2_1 *for trend*			*p<0.001*	
Ethnicity†				
Scharchop	1435	615 (42.9)	1	–
Ngalop	340	236 (69.4)	1.65	1.52–1.80
Lhotsampa	686	401 (58.5)	1.41	1.29–1.53
Khengpa	113	26 (23.0)	0.58	0.41–0.81
Other	16	9 (56.3)	1.49	0.97–2.31
Education level				
Illiterate	2372	1191 (50.2)	1	–
Literate	218	96 (44.0)	0.85	0.73–0.99
Current occupation				
Farmer/ housewife	2488	1254 (50.4)	1	–
Shopkeeper/saleswoman/manual worker	53	12 (22.6)	0.56	0.34–0.90
Clerical staff/teacher/health worker/nun	49	21 (42.9)	0.97	0.72–1.31
Marital status†				
Married/living as married	2390	1175 (49.2)	1	–

Never married	21	16 (76.2)	1.35	1.07–1.71
Widow/separated/divorced	179	96 (53.6)	1.05	0.92–1.21
Number of pregnancies†				
0	67	43 (64.2)	1.25	1.05–1.49
1–2	604	285 (47.2)	1.03	0.94–1.14
≥3	1919	959 (50.0)	1	–
χ^2_1 *for trend*			*p=0.057*	
Lifetime number of sexual partners				
0–1	2020	983 (48.7)	1	–
2	341	166 (48.7)	0.97	0.88–1.08
≥3	214	126 (58.9)	0.98	0.88–1.09
Prefer not to answer	15	12 (80.0)	–	–
χ^2_1 *for trend*			*p=0.601*	
Age at first sexual intercourse (years)‡				
9–14	245	124 (50.6)	1	–
15–16	515	235 (45.6)	0.93	0.81–1.06
17–19	968	452 (46.7)	1.00	0.89–1.12
≥20	707	365 (51.6)	1.02	0.91–1.15
Prefer not to answer / unknown	143	101 (70.6)	–	–
χ^2_1 *for trend*			*p=0.151*	
HPV infection				
Negative	2325	1168 (50.2)	1	–
Positive	265	119 (44.9)	1.01	0.91–1.13

CI=confidence interval; HPV=human papillomavirus; PR=prevalence ratio.*Adjusted for age (3 classes: 30–39; 40–49; 50+) and Basic Health Unit as appropriate. †Adjusted for age only – when adjusted for BHU, model does not converge. ‡Among sexually active women.

Table 2: Prevalence ratios (PR) for high-risk human papillomavirus (HPV) positivity and corresponding 95% confidence intervals (CI) according to selected characteristics, Bhutan, 2016

Characteristic	N tested	*care*-HPV positive N (%)	Adjusted PR*	95% CI
Age (years)				
30–39	1139	129 (11.3)	1	–
40–49	818	76 (9.3)	0.80	0.62–1.05
≥50	633	60 (9.5)	0.79	0.59–1.05
χ^2_1 *for trend*			*p=0.073*	
Region				
East	1500	173 (11.5)	1	–
West	1090	92 (8.4)	0.55	0.30–1.00
Ethnicity				
Scharchop	1435	164 (11.4)	1	–
Ngalop	340	28 (8.2)	0.68	0.31–1.49
Lhotsampa	686	58 (8.5)	0.67	0.33–1.38
Khengpa	113	15 (13.3)	1.80	0.31–10.5
Other	16	0 (0)	–	–
Education level				
Illiterate	2372	238 (10.0)	1	–
Literate	218	27 (12.4)	1.33	0.90–1.95
Current occupation				
Farmer/ housewife	2488	253 (10.2)	1	–
Shopkeeper/saleswoman/manual worker	53	3 (5.7)	0.56	0.18–1.68
Clerical staff/teacher/health worker/nun	49	9 (18.4)	1.65	0.90–3.01
Marital status				
Married/living as married	2390	232 (9.7)	1	–
Never married	21	3 (14.3)	1.53	0.54–4.38
Widow/separated/divorced	179	30 (16.8)	1.71	1.21–2.41
Number of pregnancies				
0	67	8 (11.9)	1.29	0.66–2.49
1–2	604	83 (13.7)	1.49	1.15–1.93

≥3	1919	174 (9.1)	1	–
χ_1^2 *for trend*			*p=0.007*	
Lifetime number of sexual partners				
0–1	2020	198 (9.8)	1	–
2	341	36 (10.6)	1.20	0.85–1.69
≥3	214	31 (14.5)	1.55	1.05–2.27
Prefer not to answer	15	0 (0.0)	–	–
χ_1^2 *for trend*			*p=0.022*	
Age at first sexual intercourse (years)†				
9–14	245	21 (8.6)	1	–
15–16	515	48 (9.3)	1.05	0.64–1.71
17–19	968	109 (11.3)	1.20	0.76–1.89
≥20	707	72 (10.2)	1.11	0.69–1.79
Prefer not to answer / unknown	143	14 (9.8)	–	–
χ_1^2 *for trend*			*p=0.576*	
History of PAP smear (years)				
Ever	1303	146 (11.2)	1	–
Never	1287	119 (9.3)	1.06	0.81–1.39

CI=confidence interval; HPV=human papillomavirus; PR=prevalence ratio.*Adjusted for age (3 classes: 30–39; 40–49; 50+) and Basic Health Unit as appropriate. †Among sexually active women.

Figure Legends

Figure 1: Map of Bhutan with study sites and predominant ethnic groups.*

*The size of each dot is proportional to the size of the target population of each centre.

Figure 2: Participation (%) and corresponding 95% confidence intervals by Basic Health Unit, region, and age group, Bhutan, 2016.

Figure 3: Effect of travel time (on foot) on participation in REACH Bhutan, by age group, Bhutan, 2016.

Figure 4: Percent of A) lack of previous cervical cancer screening; and B) *care*-HPV positivity by Basic Health Unit and overall in rural areas and in Thimphu, Bhutan, 2016.

*women aged 30-60 years in Tshomo et al, 2014.

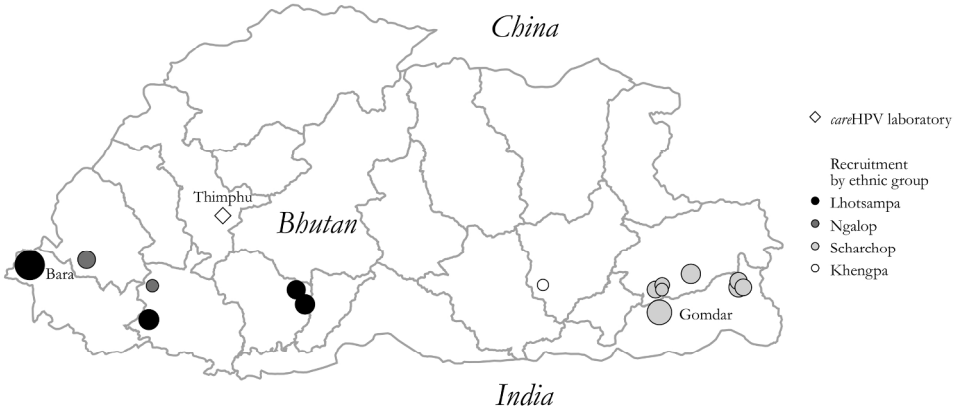

Figure 1: Map of Bhutan with study sites and predominant ethnic groups.*
The size of each dot is proportional to the size of the target population of each centre.

233x165mm (300 x 300 DPI)

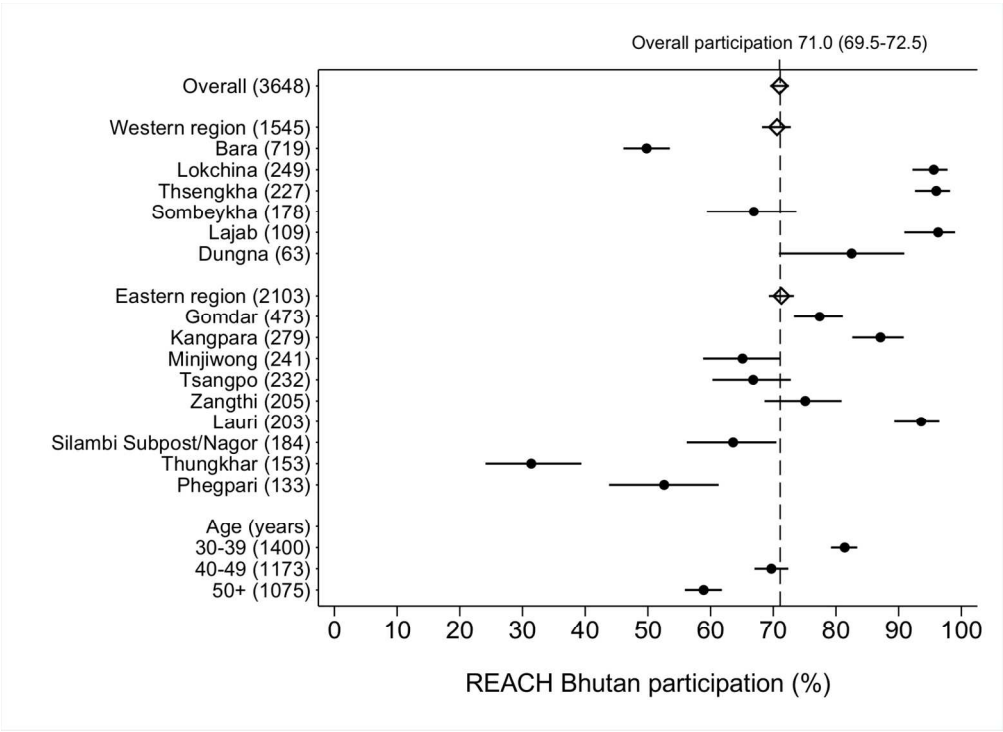

Figure 2: Participation (%) and corresponding 95% confidence intervals by Basic Health Unit, region, and age group, Bhutan, 2016.

139x101mm (300 x 300 DPI)

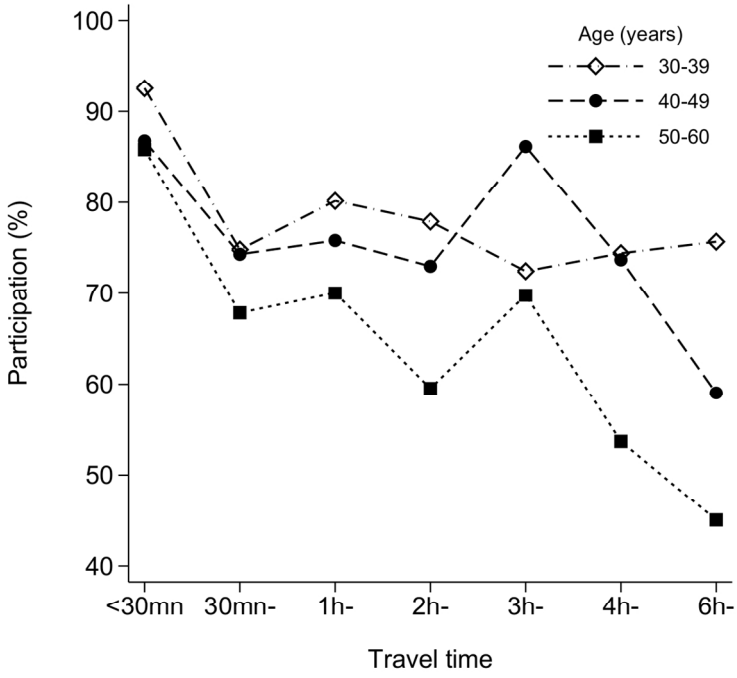

Figure 3: Effect of travel time (on foot) on participation in REACH Bhutan, by age group, Bhutan, 2016.

139x101mm (300 x 300 DPI)

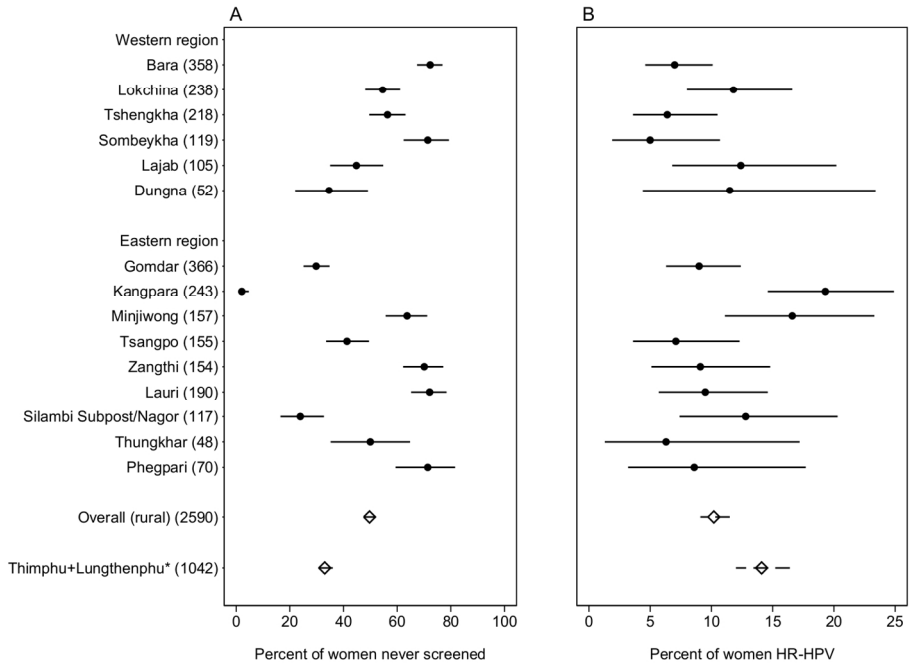

Figure 4: Percent of A) lack of previous cervical cancer screening; and B) care-HPV positivity by Basic Health Unit and overall in rural areas and in Thimphu, Bhutan, 2016.*women aged 30-60 years in Tshomo et al 2014.

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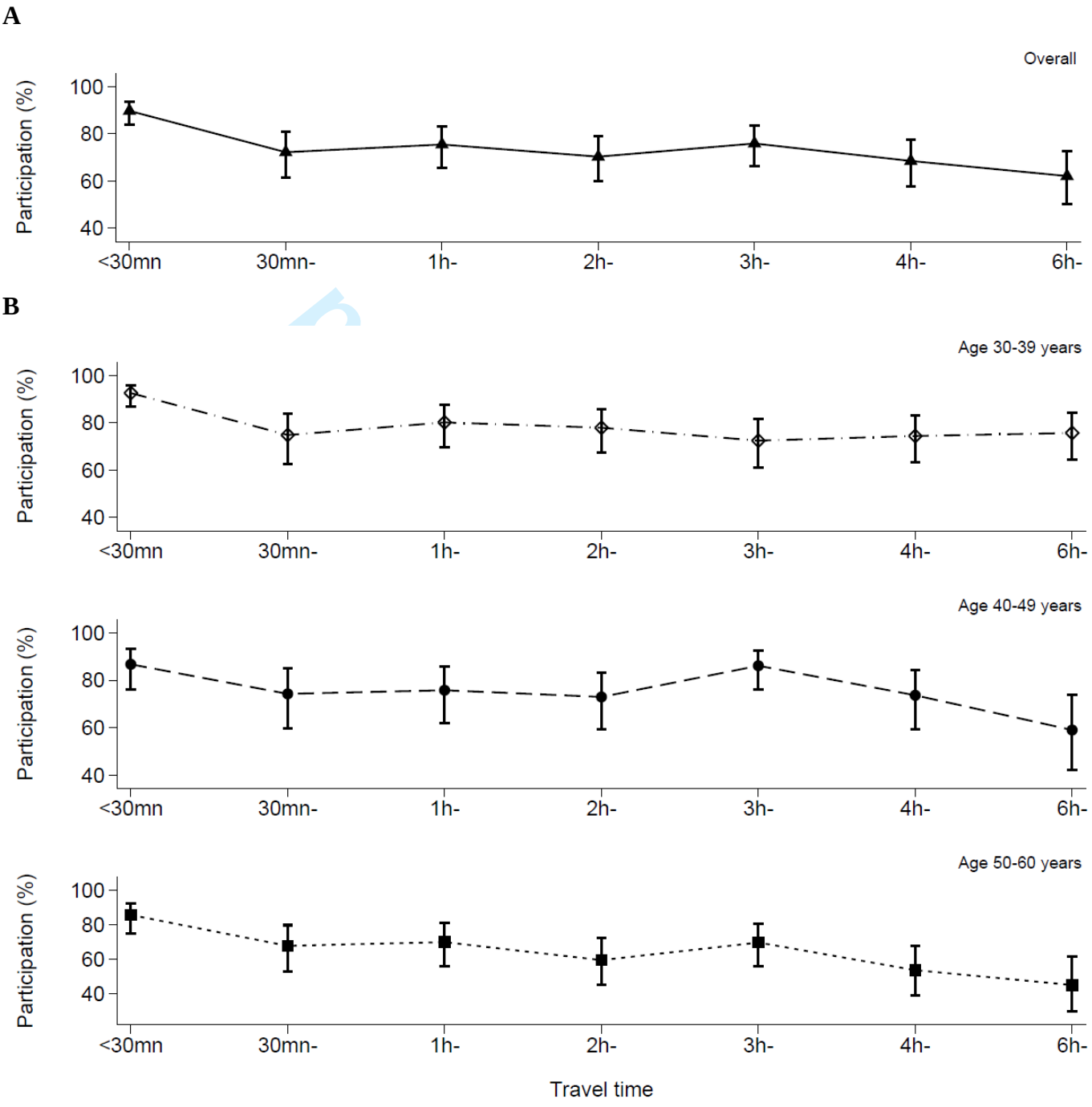
Appendix Figure 1: Effect of travel time on participation (%) in REACH Bhutan, A) overall, B) by age group with 95% confidence interval, Bhutan, 2016



STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1/3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	7
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	Fig 2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9; tables 1 & 2
		(b) Indicate number of participants with missing data for each variable of interest	9, tables 1 & 2
Outcome data	15*	Report numbers of outcome events or summary measures	9, Figure 2,4 and Tables
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted	9

		estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Tables 1 & 2
		(*b*) Report category boundaries when continuous variables were categorized	Tables 1 & 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Figure 3
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8, Figure 3
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.