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Community-based abdominal ultrasound survey of cystic echinococcosis in Bhutan: Prevalence and risk factors

Pema Wangchuk

pema.wangchuk@swisstph.ch

Swiss Tropical and Public Health Institute

Jan Hattendorf

Swiss Tropical and Public Health Institute

Jakob Zinsstag

Swiss Tropical and Public Health Institute

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Abstract

Background

Cystic echinococcosis (CE) is a neglected parasitic zoonosis, caused by larvae of the *Echinococcus granulosus sensu lato* (s.l.) complex. The parasitic transmission cycle is maintained between dog and livestock (ungulates), with humans involved as accidental hosts. In Bhutan, CE is a recognized public health and veterinary problem; however, the true burden of CE in high-risk communities remains unknown. This study assessed the community-level prevalence, potential risk factors, and awareness of CE in Bumthang District to inform CE control strategies.

Methods

We conducted a cross-sectional, community-based abdominal ultrasound survey (April–July 2025) in four gewogs of Bumthang district using multistage cluster sampling. We invited all individuals aged ≥ 12 years from the selected villages. The WHO-IWGE ultrasound staging criteria were used to classify the cysts.

Results

Among the 3,058 participants with complete data (of 3,077 screened), the overall cluster-adjusted CE prevalence (including CL) was 3.66% (95% CI: 2.92–4.59), with an ultrasound-confirmed adjusted prevalence of 2.58% (95% CI: 1.93–3.44). The liver was the most commonly affected organ (93%), and cyst stages ranged from CE1 to CE5, indicating the presence of both active and inactive infections. Intermittent dog contact (“sometimes”) was independently associated with a higher odds of prevalent CE (aOR 1.73, 95% CI 1.06–2.85). Other commonly hypothesized individual-level factors (sex, education, occupation, and place of birth) were not independently associated with infection. Incidental non-CE ultrasound findings were identified in 26% of participants, with majority being fatty liver and gallstone diseases. All participants with CE and other clinically significant ultrasound findings received counselling, stage-appropriate management for CE cases, and appropriate referral and treatment within Bhutan’s state-funded free healthcare system.

Conclusion

CE is prevalent in Bumthang district. Currently, it remains under-recognized, and data are based solely on hospital-based passive case detection. The pattern of modest village-level clustering and weak discrimination by individual-level risk factors is consistent with the “environmental-borne” transmission hypothesis and community-level exposure risk described in recent molecular and ecological studies. These findings support One Health control strategies focused on sustained dog deworming, safe offal

and slaughter practices, improved environmental hygiene, and geographically informed integrated surveillance rather than targeted approaches for high-risk groups.

Introduction

Background

Cystic echinococcosis (CE) is caused by the larval stage of the *Echinococcus granulosus sensu lato* (s.l.) complex. CE remains a globally significant zoonotic disease that affects both human and animal health, particularly pastoral and livestock rearing communities [1]. Despite sustained control efforts, CE continues to pose a major public health and economic burden in many endemic regions, including Central Asia, South America, parts of Africa, and the Himalayas [2]. By 2030, the number of global incident CE cases is projected to increase from ~207,000 (2019) to ~236,000 (13.7%) [3]. The World Health Organization (WHO) recognizes CE as a neglected tropical disease (NTD) and a high-burden foodborne parasitic disease, with a high disability attributable to its chronic nature and the need for surgical intervention for active disease [4,5].

CE is closely associated with livestock rearing, the presence of free-roaming dogs, and poor hygiene and sanitation practices [6]. In pastoral communities where dogs have close contact with livestock, humans are primarily exposed to *Echinococcus* infections through contaminated food and water [7]. A Recent One Health study from South Asia highlighted that low awareness, poor hygiene, and informal slaughtering practices significantly increase the risk of exposure [8].

Bhutan's agropastoral and socio-cultural context is characterized by scattered and sparse rural settlements, free-ranging livestock grazing, and close dog-human interactions [9–11], factors which facilitate parasite persistence and transmission. A recent retrospective hospital-based study documented the burden and spatial distribution of CE in Bhutan, indicating the need for improved disease surveillance and control measures [12]. Molecular analyses conducted by the National Center for Animal Health have confirmed the presence of *E. granulosus sensu stricto (s.s.)* (G1-3 genotypes) and *E. ortleppi* (G5 genotype) in cattle, a dominant livestock species in the country, and dogs, indicating active transmission cycles among domestic hosts [13,14]. Although human infection due to *the E. ortleppi* genotype is rare, the emergence of human cases in Asia, including South Asia, has been reported [15]. A hospital-based study that reported cases from multiple districts across the country and molecular confirmation of the parasite's presence in the environment indicates silent community transmission. Most cases remain underdiagnosed because of the lack of disease surveillance and diagnostic infrastructure, particularly in rural areas [12,16].

To date, epidemiological data on CE in Bhutan have been mainly derived from hospital-based records and isolated case reports. No population-based ultrasound surveys have quantified the prevalence of CE or evaluated exposure patterns in the country. Understanding the epidemiology of CE in Bhutanese communities is critical for developing effective One Health control strategies, including the integration of

CE surveillance into broader ‘priority-zoonotic disease’ surveillance and control programs. This study aimed to determine the community prevalence of CE and its associated risk factors in a high-risk district, fill existing knowledge gaps, and contribute evidence toward informing national CE control strategies.

Materials and Methods

Study Design and Setting

This cross-sectional, community-based study was conducted between April and July 2025 in Bumthang district. As shown in Fig. 1, Bumthang district is in the north-central region, situated at approximately 27.5°–27.9° N and 90.6°–91.1° E latitudes. The district has four major river valleys, characterized by high-altitude Himalayan ecology, with elevations ranging between 2,600 and 4,500 meters above sea level. The majority of residents’ livelihoods are largely based on subsistence agriculture, livestock farming, and seasonal pastoralism, particularly yak and cattle herding at higher elevations during warmer summer months. Bumthang has a predominantly rural population of approximately 17,000 residents, with scattered settlements and a relatively low population density [17].

Bhutan has a three-tiered, state-funded health system in which the state provides free healthcare services to its citizen, including the full cost of ex-country referral for treatment that is not available in the country [18]. In line with the country’s health service delivery, health services in Bumthang are delivered through one district general hospital (Wangdicholing Hospital), five primary health centers (PHCs), and outreach clinics that serve remote communities across four gewogs (administrative blocks). Access to diagnostic imaging (ultrasonography) services is limited outside the district hospital catchment area, particularly in rural and semi-nomadic areas, making Bumthang a relevant setting for community-based ultrasound screening for CE.

The study sites comprised rural areas of four gewogs, selected as pilot sites due to previously reported higher CE burden/cases from hospital reports. The Chamkhar town area and the catchment population of the district hospital were excluded because of their routine access to diagnostic ultrasound services. Participants were identified from PHCs registries and recruited in collaboration with local (*chiwog* and village-heads) leaders, including a limited number of semi-nomadic pastoral households engaged in yak herding in the district’s northern region.

Study Population, sample size and sampling

The target population included permanent residents of selected *chiwogs* and villages in the Bumthang district, including semi-nomadic pastoralist communities. Eligible participants were individuals aged ≥ 12 years who had resided in the selected households for at least six months, provided informed consent (or assent for minors), and were present during the survey. Individuals with acute or severe illnesses that prevented attendance and temporary residents were excluded.

The sample size was calculated to estimate the CE prevalence with a precision of two percentage points (half-width of the 95% CI), assuming a prevalence of 4% and an intraclass correlation coefficient of 0.05. With an average cluster size of 30 individuals, the corresponding design effect was estimated to be 2.45. We estimated that we required a minimum of 38 clusters and 1,140 individuals and inflated this number by 10% to account for non-response [19]. Sampling followed a three-stage cluster design using probability proportional to size (PPS). In the first stage, 15 of the 20 *chiwogs* were selected by PPS based on the number of households. In the second stage, approximately three villages were selected as primary sampling units (PSU) per *chiwog* (44 villages total). In the final stage, all households within the selected villages were included, and all eligible members of the selected households were invited to undergo ultrasound screening and complete questionnaires. In total, 3,077 individuals from 44 villages participated, reflecting high participation rates and the inclusion of all eligible individuals from the selected villages.

Data Collection Procedures & Quality Assurance

Ultrasound examinations and questionnaire administration were conducted at the respective PHCs or community centers, depending on the participants' accessibility. Abdominal ultrasound examinations were performed using Mindray M6 and MX7 machines with curvilinear transducers in the conventional B-mode, following a standardized scanning protocol. Abdominal ultrasound is the recommended first-line diagnostic tool for hepatic CE, with high reported diagnostic performance in experienced hands, particularly for active cyst stages (CE1-CE3), based on comparative imaging studies and expert consensus [20–22]. Examinations were performed by the principal investigator and an experienced (> 10 years) medical sonographer from the Wangdicholing District Hospital. Data on sociodemographic characteristics, dog-related practices, food handling and hygiene behaviors, environmental exposures, and awareness of CE were collected using a structured questionnaire developed specifically for this study (Supplementary Table S3). Questionnaire was pilot tested in 2 non-study villages and items were revised accordingly for clarity, length and appropriateness prior to the main survey. Participants completed the questionnaire before the ultrasound examination.

As part of the data quality assurance, sonographer and data collectors/field staff were trained on the study protocol, and briefings were provided before each session. A subset of ultrasound images of CE cysts was independently double-read to assess inter-observer agreement. Field supervisors and the principal investigator checked the completeness and consistency of the questionnaires daily on Open Data Kit (ODK) Collect. After verification, complete questionnaire forms were submitted to ODK Central. Data validation rules were applied during data entry in the ODK collect forms.

Data Management and Statistical Analysis

Data were downloaded from ODK Central as comma-separated value files and imported into R software for cleaning and analysis. The datasets were checked for completeness and internal consistency. Range and logic checks were applied to identify outliers and implausible values in the data. Duplicate records were removed, variables were re-coded as required, and the distributions of continuous variables (age

and cyst size) were examined and appropriately described. All analyses were conducted using R software (version 4.4.3).

Case Definition and Study Variables

Confirmed CE cases were defined as 'individuals with ultrasound findings pathognomonic of echinococcal cysts', and suspected cases were defined as 'ultrasound findings consistent with, but not pathognomonic of CE cysts.' To avoid systematic underestimation, suspected cases included cystic lesions (CL), which are considered 'potentially echinococcal in the absence of advanced imaging or serology' [21, 23]. Both confirmed and suspected cases were combined into CE cases, and a single binary outcome (CE vs. non-CE) was used in all analyses. Socio-demographic variables included sex and age, which was categorized as < 19, 20–39, 40–59, and ≥ 60 years. Other categorical variables included educational attainment, occupation, and place of birth (Bumthang natives versus other districts). Behavioral exposures included frequency of dog contact as a categorical exposure variable, raw meat consumption (yes/no), and handwashing (always/often, sometimes/rarely/never).

Among the CE cases, the cyst stage was classified according to the WHO-Informal Working Group on Echinococcosis (WHO-IWGE) ultrasound classification. The cyst stages and cyst activity were analyzed and described at the cyst level. Person-level summaries were derived for the number of cysts, cyst location, and cyst diameter.

Statistical analysis: risk factor modelling and model selection

Prevalence estimation

Prevalence analyses were based on all screened individuals with complete ultrasound results and village identifiers (n = 3,058). The prevalence of confirmed, suspected, and overall CE (confirmed and suspected) was calculated as the proportion of cases among those screened with complete data. To account for the clustered sampling design, we used intercept-only logistic regression models with cluster-robust standard errors (sandwich estimator) and clustering adjustment at the village level. This approach avoids the instability of mixed-effects models for rare outcomes while properly adjusting for within-village correlation. The point prevalence was obtained by back-transforming the model intercept to the probability scale using the inverse logit function as follows

$$\text{Prevalence} = \exp(\beta_0) / (1 + \exp(\beta_0))$$

Cluster-robust 95% confidence intervals were calculated on the log-odds scale and then back-transformed to the probability scale.

Bivariate analysis

Bivariate and risk factor analyses were restricted to participants with complete covariate data (n = 3,058). The associations between CE and each explanatory variable were assessed individually using mixed-effects logistic regression models with a village-level random intercept. Crude odds ratios and 95% Wald confidence intervals were estimated using these models. For each variable, a global p-value was obtained from a likelihood ratio test (LRT) comparing the model with the predictor to the intercept-only model using the drop1() function. Variables with a global LRT p-value < 0.25 were considered candidates for the multivariable model to avoid excluding potentially important confounders. Age group and sex were retained a priori, regardless of their bivariate p-values.

Multivariable modelling

A multivariable mixed-effects logistic regression model with a village-level random intercept was fitted to estimate adjusted odds ratios (aORs) and 95% confidence intervals. The model performance was assessed using convergence diagnostics and singularity checks (no singular fit was detected). The intraclass correlation coefficient (ICC) was calculated from the village-level random-effects variance as $\sigma^2_u / (\sigma^2_u + \pi^2/3)$. As a sensitivity analysis, alternative clustering structures were explored, including household-level clustering and households nested within the villages. These models were compared using the Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and likelihood ratio tests. The village-only random intercept model was retained as the primary analysis model because household-level clustering was statistically unstable due to sparse binary outcomes and a median household size of two. All analyses were conducted using R software (version 4.4.3), with mixed-effects models fitted using the *lme4 package*, model diagnostics assessed using the performance package, and tables prepared using the *gtsummary* package.

This study was reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for cross-sectional studies [24] (Supplementary Table S4).

Ethical Considerations

All study procedures during the survey were conducted in accordance with the Declaration of Helsinki. The study protocol, which included a prespecified ancillary care plan within the context of Bhutan's free health system, was reviewed and approved by the Research Ethics Board for Health, Ministry of Health, Bhutan (approval number: *Ref. No./PO/RL/2024.1.NNW*). In addition, the study protocol was reviewed by the Ethikkommission Nordwest-und Zentralschweiz, Switzerland (Study ID: *AO_2024_00045*). Informed consent was obtained from all adult participants, and informed parental or legal gaurdians' consent and informed assent for minors (< 18 years) after explaining the study objectives, procedures, risks, benefits and voluntary participation orally in Dzongkha or the local dialect (*Bumthap)*. For participants who were not abel to read, the information sheet (Dzongkha version) was read aloud in the presence of a witness, and informed consent was documented by a thumb impression. Participants with clinically significant ultrasound findings, including CE, were treated, counselled on their diagnoses and referred to the nearest health facility for further evaluation and management. All required additional diagnostic work-up and

treatment were provided within the country's state-funded, free healthcare system, at no cost to the patients. All data were pseudo-anonymized and handled confidentially.

Ancillary care

The study team counselled and, where necessary, referred every participant with a clinically significant ultrasound finding for further evaluation and management at the nearest health facility. The participants were issued a written clinical summary. In addition, their clinical information was entered in the national Electronic Patient Information System (ePiS), and each referred case was tracked to a documented clinical contact.

Confirmed and suspected CE cases

All participants with ultrasound-confirmed CE cysts were staged using the WHO-IWGE standardised ultrasound classification and managed according to stage-specific recommendations [25]. Individuals with active cysts received antiparasitic therapy after baseline blood work-up, and were referred for surgical management when indicated, while those with inactive cysts (CE4 and CE5) were managed following watch-and-wait protocol. Participants with suspected CE cysts (cystic lesions) were referred to referral hospitals for confirmatory imaging. All confirmed and suspected CE cases were entered into a follow-up registry shared with the respective catchment PHCs to facilitate longitudinal monitoring and continuity of care.

Incidental non-CE findings

Participants with clinically significant non-CE findings received on-site counselling and referral to the nearest health center as appropriate. Individuals with sonographic fatty liver disease were provided lifestyle counselling aligned with the WHO Package of Essential Noncommunicable Disease interventions (WHO PEN) [26]. In addition, these individuals were enrolled in the Service with Care and Compassion Initiative (SCCI) registry at their respective PHC for NCD screening, brief intervention for alcohol consumers and follow-up. Participants with symptomatic gallstone disease or other significant conditions were referred to district or referral hospitals for further evaluation and management.

Results

Study Population Characteristics

Of the 3,077 screened participants, 3,058 with complete data were included in the prevalence and risk factor analyses. Participants were from 1266 distinct households across 44 villages from four gewogs of Bumthang district. The median household size was two members (IQR: 1–18). More than half (60%) of the study participants were female (1820/3058). The study population had a median age of 43 years (IQR: 30–59 years). Approximately one-third of the study participants were aged 20–39 years (31%) and 40–59 years (32%), while 24% were aged ≥60 years and 13% were aged <19 years.

As shown in Table 1, 82% of the study participants were natives, and the remaining (18%) migrated from other districts. Among participants not native to Bumthang, 73% (n = 376) had lived in the district for more than five years, while 15% (n = 79) reported a duration of stay between one and five years, and 12% (n = 62) had lived there for less than one year. The occupational and educational characteristics of the study participants are presented in Supplementary Table S1.

Table 1: Baseline demographic characteristics of the study participants.

Characteristic	*N*	*n (%)*
Sex	3,058	
Male		1,238 (40%)
Female		1,820 (60%)
Age Group	3,058	
<19		411 (13%)
20-39		937 (31%)
40-59		986 (32%)
60+		724 (24%)

Prevalence of Cystic Echinococcosis

Confirmed and suspected CE (including CL) were combined for prevalence estimation, consistent with the WHO Informal Working Group on Echinococcosis (IWGE) recommendations. Among the 3058 screened individuals, 112 were CE-positive (79 confirmed and 33 suspected). After adjusting for village-level clustering, the overall adjusted prevalence of CE was 3.7% (95% CI: 2.9–4.6), cluster-adjusted prevalence of confirmed CE was 2.58% (95% CI: 1.93–3.44), and cluster-adjusted prevalence of suspected CE was 1.08% (95% CI: 0.76–1.53). Of the 79 confirmed CE cases, 26.6% (21/79) had been previously diagnosed.

The overall prevalence and age group-wise estimates are presented in Figure 2. The prevalence of CE increased markedly with age, rising from 1.88% among individuals aged <19 years to 6.57% among those aged ≥60 years. In terms of sex, the prevalence was higher among females (4.2%) than among males (2.9%). Individuals with no formal education had the highest prevalence (4.4%), whereas a lower prevalence was observed among those with secondary or higher education. By occupation, farmers had the highest prevalence (4.3%), followed by students (1.7%) and other occupational groups. The prevalence was higher among individuals native to Bumthang District (3.6%) than among those born in other districts (1.7%).

Incidental (non-CE) ultrasound findings

Incidental non-CE findings were detected in 796 participants (26%). In 26 cases, specific incidental findings were not recorded. Among 770 with recorded incidental findings, sonographic fatty liver disease accounted for 85.6% (n=659) of these cases, followed by gallstone disease (cholelithiasis) at 9.9% (n=76). Less frequent findings included simple renal cysts at 2.1% (n=16), hepatic haemangioma at 0.8% (n=6), renal stones at 0.4% (n=3), hydronephrosis at 0.4% (n=3), and ovarian cysts at 0.3% (n=2). Among participants with fatty liver, Grade I steatosis predominated (59.0%, n=389), followed by Grade II (28.4%, n=187) and Grade III (0.9%, n=6); a further 11.7% (n=77) had fatty liver grading was not recorded. As alcohol consumption data were not collected at the time of ultrasound examination, differentiation between alcoholic and non-alcoholic fatty liver disease was not possible.

Geographic Distribution and Awareness

As shown in Figure 3, the cluster-adjusted gewog-wise CE prevalence varied across the four gewogs in the district, ranging from 1.39% in Tang gewog to 4.92% in Chhoekhor gewog. The highest number of CE cases was detected in Chumey (47/1254), with a cluster-adjusted prevalence of 3.65% (95% CI: 2.28–4.89), whereas the lowest number of cases was detected in Tang (8/577), indicating modest within-village heterogeneity and focal endemicity in the district.

Awareness of CE and reporting of a family member affected by CE showed only modest differences between the CE and non-CE cases. Overall, disease awareness of CE and family history of the disease were not frequently reported in the study population.

CE Cyst Characteristics

Among individuals with CE cysts, the most frequent WHO ultrasound stages at the cyst level were CE1 (26.4%) and cystic lesions (CL) (23.6%), followed by CE3B (18.2%) and CE5 (10.9%) stages. A total of 116 cysts were detected among 112 individuals; nine individuals had two or more cysts each, resulting in cyst-level totals that exceeded person-level counts. Of these, 110 cysts were classified according to the WHO-IWGE staging system. At the cyst level, 56.4% of the cysts were active (CE1-CE3b), 20% were inactive (CE4 and CE5), and 23.6% were CL lesions. Of the 104 individuals with a known cyst number, most had a single cyst (91.3%), and the rest had multiple cysts (≥ 2). The liver was the predominant site of infection (93%), and extrahepatic involvement was observed in a small proportion of patients. Regarding cyst size, approximately half of the individuals had the largest cyst measuring 5–9.9 cm, while 36.8% had cysts smaller than 5 cm, and 12.3% had cysts ≥10 cm in diameter.

Risk Factor Analysis

The crude and adjusted associations of sociodemographic and behavioral factors with CE are shown in Table 2. Crude and adjusted odds ratios were estimated using mixed-effects logistic regression with a village-level random intercept. The per-category Wald p-values were presented in Table 2. Numbers (N) vary by variable due to missing data; multivariable models were fitted using complete-case data (n = 2,842). Handwashing and raw meat consumption were not associated with CE infection (Supplementary Table S2). The multivariate results are discussed below.

Table 2. Bivariate and multivariate mixed-effects logistic regression analysis for CE

Characteristic	N	n CE (%)	Crude OR (95% CI)	aOR (95% CI)	p
Sex					
Male	1,238	36 (2.9)	—	—	
Female	1,820	76 (4.2)	1.45 (0.97–2.16)	1.20 (0.75–1.91)	0.50
Age group (years)					
<19	411	7 (1.7)	—	—	
20–39	937	16 (1.7)	0.95 (0.39–2.32)	1.34 (0.22–8.29)	0.80
40–59	986	38 (3.9)	2.21 (0.98–5.00)	2.10 (0.33–13.5)	0.40
60+	724	51 (7.0)	4.82 (1.92–9.52)	4.27 (0.64–28.2)	0.13
Education					
No formal education*	1,259	55 (4.4)	—	—	
Non-formal education**	119	5 (4.2)	1.04 (0.40–2.68)	1.34 (0.50–3.56)	0.60
Primary level	407	14 (3.4)	0.76 (0.42–1.38)	1.29 (0.62–2.66)	0.50
Secondary/High school	746	8 (1.1)	0.23 (0.11–0.49)	0.42 (0.17–1.06)	0.06
Diploma/College & Above	154	5 (3.2)	0.71 (0.28–1.80)	1.01 (0.27–3.72)	>0.90
Monastic education	157	5 (3.2)	0.71 (0.28–1.80)	1.14 (0.39–3.33)	0.80
Occupation					
Farmer	2,139	91 (4.3)	—	—	
Student	423	7 (1.7)	0.39 (0.18–0.85)	1.22 (0.21–7.01)	0.80
Businessperson	106	3 (2.8)	0.65 (0.20–2.10)	1.48 (0.43–5.05)	0.50
Civil servant	100	3 (3.0)	0.69 (0.21–2.20)	2.10 (0.50–8.79)	0.30

Monk/nun/monastic/military	70	1 (1.4)	0.33 (0.05–2.39)	0.40 (0.05–3.50)	0.40
Others	220	7 (3.2)	0.72 (0.33–1.58)	1.40 (0.59–3.29)	0.40
Place of birth					
Native of Bumthang	2,325	83 (3.6)	—	—	
From other districts	516	9 (1.7)	0.48 (0.24–0.96)	0.51 (0.24–1.08)	0.08
Dog contact frequency					
Never	1,813	60 (3.3)	—	—	
Rarely	168	3 (1.8)	0.56 (0.17–1.70)	0.59 (0.18–1.92)	0.40
Sometimes	651	27 (4.1)	1.31 (0.82–2.10)	1.73 (1.06–2.85)	0.03
Often	114	1 (0.9)	0.27 (0.04–1.95)	0.32 (0.04–2.34)	0.30
Always	96	1 (1.0)	0.32 (0.04–2.32)	0.38 (0.05–2.80)	0.30

** No formal education* refers to individuals who have not attended formal schooling and are illiterate. *Non-formal education*** refers to individuals who received structured functional literacy, numeracy, or life-skills training, who missed the formal school system.

In the multivariable multi-level logistic regression analysis (Table 2), secondary/high school education, non-native residency, and “sometimes” dog contact were the only factors associated with CE. However, only intermittent (“sometimes”) dog contact was statistically significant after adjusting for other variables. The association between intermittent dog contact (“sometimes”) and prevalent CE strengthened after multivariable adjustment: the crude OR (village random-intercept model) was 1.31 (95% CI: 0.82–2.10, p = 0.30), whereas the adjusted OR increased to 1.73 (95% CI: 1.06–2.85, p = 0.03), a 32.1% change in the odds ratio. This change is consistent with negative confounding by age, education, and occupation, which differ markedly between the “never” and “sometimes” contact groups: the “never” group was older (26.3% aged ≥60 vs. 14.7%), had less formal education (50.4% vs. 29.2%), and comprised more farmers (74.6% vs. 53.8%)—all factors independently associated with higher CE prevalence. Their confounding effect inflated the baseline risk in the reference group, thereby attenuating the crude contrast.

Discussion

Main Findings

This community-based ultrasound survey, the first of its kind in Bhutan, screened 3,077 individuals aged > 12 years across 44 villages in Bumthang district. The overall cluster-adjusted CE prevalence (including CL) among 3058 individuals with complete data was 3.7% (95% CI: 2.92–4.59). Among them, 2.6% had ultrasound-confirmed CE. Most cases were not previously diagnosed. The prevalence we observed falls within the range reported from comparable endemic settings in the region: 1.7% in the Tibet Autonomous Region [27], 0.7% among nomadic communities on the Qinghai-Tibet Plateau [28], and 4.9% in rural Mongolia [6]. In contrast, hospital-based estimates from Bhutan reported an annual incidence of approximately 4.4 per 100,000 between 2014 and 2019 [12], which is significantly different. This is consistent with the well-documented gap between passive case detection and the true community burden in endemic regions [16, 21]. This finding confirms that CE is a substantial yet under-recognized public health problem in Bumthang and, at large, in the whole country, where facility-based passive surveillance captures only a fraction of the true disease burden.

In addition, our findings from the risk factor analysis support the 'environmental-borne' transmission hypothesis in endemic settings, where exposure risks are widespread at the community level. The weak discrimination by individual-level factors is consistent with recent molecular and ecological evidence from South America, Iran, and China, which showed that *Echinococcus* contamination levels can be similar in households with and without detected cases [29, 30]. In such settings, interventions targeting only dog-owning households or specific occupational groups are unlikely to be sufficient; integrated One Health strategies, such as sustained dog deworming, safe offal disposal, improved slaughter hygiene, and community-wide health education are required [16, 29].

Cyst Characteristics

Among the 112 individuals with CE, the liver was affected in 93.0% of the cases, consistent with the known organotropism of *E. granulosus* [21, 27]. Based on the WHO-IWGE staging, 56.4% of the staged cysts were active (CE1, CE2, CE3a, and CE3b), 20% were inactive (CE4 and CE5), and 23.6% were classified as CL or non-classifiable. Most individuals (91.3%) had a single cystic lesion. Cyst sizes ranged from < 5 cm (36.8%) to ≥ 10 cm (12.3%). The detection of active CE1 cysts indicates ongoing community transmission [23]. The case mix of active and inactive stages across age groups also indicates long-standing infections accumulated over years of environmental exposure, a pattern reported in similar community surveys from Uzbekistan, Chile, and Mongolia [6, 29, 31].

The proportion of active cysts is clinically important because these cyst stages harbor viable protoscoleces and typically require intervention based on the cyst stage, such as albendazole, percutaneous treatment, or surgery, depending on the cyst characteristics [21, 25]. The co-existence of inactive cysts in approximately one-fifth of cases, likely representing spontaneous cyst involution, adds to the overall disease burden and requires ongoing surveillance [25, 31]. The fact that most cases were previously undiagnosed suggests the need for active community screening, particularly given recent

environmental studies showing widespread contamination, even in households without detected cases [30, 32].

Risk Factors

Dog contact frequency showed no significant gradient with CE prevalence: 3.3% (never), 1.8% (rarely), 4.1% (sometimes), 0.9% (often), and 1.0% (always). The adjusted odds ratio for “sometimes” contact reached statistical significance (aOR 1.73, 95% CI: 1.06–2.85, p = 0.03), whereas all other categories showed no association. The strengthening from the non-significant crude OR of 1.31 (95% CI: 0.82–2.10) shows negative confounding. The “never” reference group constituted older, less educated, and farmer by occupation than the “sometimes” group; the characteristics that highly independently increase the risk of CE infection, and therefore, inflated the baseline prevalence in that group, suppressing the true effect in the unadjusted analysis [33, 34]. The absolute prevalence differences across groups were small, and the isolated “sometimes” signal did not support a meaningful graded exposure effect. Self-reported dog-contact frequency is a coarse proxy for fecal-oral transmission risk in settings where free-roaming dogs are ubiquitous and environmental contamination is widespread [6, 7, 35]. This finding likely reflects residual confounding or exposure misclassification rather than a distinct biological pathway and should be interpreted with caution.

In addition, age showed a consistent upward gradient in point estimates, from aOR 1.34 (20–39 years) to 4.27 (≥ 60 years) relative to those < 19 years, but none reached statistical significance, likely owing to limited power, with 112 cases distributed across multiple strata. The direction of the gradient is consistent with CE's chronic natural history of CE, in which prevalence reflects cumulative lifetime exposure rather than recent infection [22]. Population surveys in Tibet have documented a statistically significant positive age-prevalence trend across > 80,000 individuals [27], supporting the biological plausibility of our findings, even though we lacked the power to confirm this. However, age represents cumulative exposure rather than a modifiable risk factor for CE.

In terms of risk factors, several commonly hypothesized risk factors, such as sex, occupation, educational attainment, and frequent dog contact, were not independently associated with prevalent CE after adjustment for confounding factors. This pattern requires careful interpretation. Farmers constituted 70% of our study population, with a crude prevalence of 4.25%; however, occupation was not a significant predictor in the adjusted model. Similarly, individuals with no formal education had a crude prevalence of 4.37% compared with 1.07% among those with secondary or higher education; however, this association was attenuated after adjustment (aOR 0.42, 95% CI: 0.17–1.06, p = 0.065), suggesting a protective effect that did not reach significance. Both patterns have been reported elsewhere: in parts of Tibet, occupational gradients are pronounced–3.66% among herdsmen vs. 1.66% overall [27], but in mixed farming communities like Bumthang, where livestock rearing is near-universal and free-roaming dogs are ubiquitous, occupational categories lose their discriminatory value. Community surveys in Mongolia and the Yunnan province have similarly found education to be protective in multivariable

models [6, 36], supporting the idea that education acts as a structural factor that shapes long-term behavior rather than conferring immediate protection.

Although not significant in the adjusted model, individuals born outside Bumthang had lower adjusted odds of CE (aOR 0.51, 95% CI: 0.24–1.08, p = 0.08). This is consistent with the cumulative exposure effect, in which long-term residence in an endemic environment increases the likelihood of infection over time. More detailed migration histories, including age at arrival and duration of childhood exposure, are needed to disentangle this issue [21].

Village-Level Clustering and Spatial Variation

The ICC of approximately 3.3% indicates a small but meaningful fraction of the variation in CE occurrence attributable to inter-village differences. The prevalence ranged from 1.39% in Tang to 4.92% in Chhoekhor gewog, consistent with the focal transmission heterogeneity reported across Himalayan and Central Asian settings [27, 28, 35]. On the Qinghai-Tibet Plateau, smaller and more remote settlements have a higher CE prevalence linked to a greater distance from township centers and a higher dog density [28]. In the Kyrgyz Republic, community-level risk profiles varied substantially between villages, even within the same region [36].

The modest ICC suggests that while local ecological factors (dog density, livestock management, altitude, and water sources) contribute to heterogeneity, exposure is broadly distributed across the district rather than confined to a few highly clustered villages. This is consistent with transmission maintained by environmental persistence and the dispersal of *Echinococcus* eggs across the landscape by stray dogs [37]. Spatial analyses incorporating canine surveillance and environmental sampling data would help identify higher-burden zones/areas for targeted interventions within Bumthang.

Strengths and Limitations

This study is the first large-scale community-based CE ultrasound survey in Bhutan, with a sample of 3,077 individuals across all four gewogs of Bumthang district, which is comparable in scale to recent surveys in Mongolia, Uzbekistan, and the Qinghai-Tibet Plateau [6, 28, 35]. We used the standardized WHO-IWGE ultrasound classification and applied two complementary approaches to account for the clustered sampling design: intercept-only logistic regression with cluster-robust standard errors (sandwich estimator) for prevalence estimation and mixed-effects logistic regression with village-level random intercepts for risk factor analyses, in line with the best practices for community-based surveys [38].

Nevertheless, this study has several limitations. The cross-sectional design precludes causal inference because CE is chronic and long-latent, and prevalent cases reflect cumulative past exposure, making it difficult to link current behaviors to infection status [39]. Key exposures were self-reported and subject to recall and social desirability bias; misclassification of complex exposures, such as dog contact, may have attenuated true associations toward the null hypothesis [36]. The relatively small number of CE

cases (n = 112) limited the statistical power (wide confidence intervals), particularly for detecting associations across multiple categorical strata. In addition, residual confounding by unmeasured environmental exposure cannot be excluded. Therefore, non-significant results should not be assumed to represent the true null effects. Although abdominal ultrasound is the most practical field screening tool, it may miss very early, heavily calcified, or extra-abdominal diseases, slightly underestimating the true burden [40]. Additionally, cystic lesions (CL), sonographically indeterminate cysts that cannot be definitively assigned to a WHO-IWGE stage, were included in the overall prevalence estimate, in line with the WHO-IWGE guidance [21], as they are considered potentially echinococcal. However, CL may not represent true CE, and their inclusion (26/116 cysts, 23.6%) may overestimate the true CE burden; the prevalence estimate restricted to confirmed CE cases (CE1–CE5 stages) was 2.58% (95% CI: 1.93–3.44). Finally, questionnaire-based measures may not adequately capture exposure to environments with widespread contamination across multiple matrices [30, 37]. The integration of dog fecal testing and molecular detection of *Echinococcus* eggs in the environment (soil and water) would provide more objective community-level exposure measurements [30].

While these findings provide a solid baseline for the Bumthang district, extrapolation to other parts of Bhutan should be cautiously considered despite the country's ecological and socio-cultural similarities. Nonetheless, expanding community-based ultrasound surveillance to other endemic districts would enable a more comprehensive national picture and support geographically targeted control strategies for the disease.

Implications for One Health Policy and Practice

Several findings from this survey have direct implications for CE control in Bhutan and comparable endemic settings. The large gap between our community-level estimate (3.66%) and the previously reported hospital-based incidence of 4.4 per 100,000) [12] makes a strong case for integrating periodic community-based ultrasound screening into routine public health activities in endemic districts. While our primary estimate includes suspected cases (CL), in line with the WHO-IWGE guidelines, the confirmed CE prevalence of 2.58% provides a more conservative baseline for resource allocation, highlighting the need for careful consideration in programmatic planning. Most of the 112 CE cases we identified were previously undiagnosed, and more than half had active cysts that required clinical intervention. In a setting where CE is chronic and largely asymptomatic, passive facility-based surveillance alone will continue to miss the majority of infections, delaying treatment and obscuring the true scale of the problem. Establishing referral pathways that link community screening to district- and higher-level clinical management, including implementation of cyst staging, and stage-based treatment approaches based on international guidelines, would be a practical first step.

The finding that intermittent dog contact was the only independently associated risk factor, while frequent contact, dog ownership, occupation, and education were not, argues against control strategies that target narrowly defined "high-risk" groups. In Bumthang, where free-roaming dogs are common and environmental contamination appears widespread, exposure is shared across the community rather than

concentrated in particular households or occupations. This supports population-level rather than individually targeted interventions: sustained, systematic dog deworming programs covering community and stray dogs, not only owned animals, combined with improved slaughterhouse hygiene, safe offal disposal, and regulation of home slaughter practices. The modest village-level clustering (ICC ~ 3.3%) further supports district-wide rather than village-specific roll-out, although the observed gewog-level variation (1.39%–4.92%) suggests that higher-burden areas such as Chhoekhor gewog could be prioritized for initial implementation.

The coexistence of active and inactive cyst stages across age groups indicates that transmission has been sustained over a prolonged period. This highlights the need for long-term inter-sectoral programmatic commitment, regular dog deworming cycles, ongoing community education, and structured follow-up programs to monitor the impact. The upward age gradient in prevalence, although not statistically significant in our data, is consistent with cumulative lifetime exposure and suggests that even modest reductions in environmental contamination could yield measurable benefits over time, particularly among younger cohorts with less cumulative exposure.

In addition, the limitations of questionnaire-based exposure assessment in our study and CE epidemiology more broadly highlight the value of integrating environmental and veterinary surveillance into a One Health monitoring framework. Coproantigen testing of dogs, molecular detection of *Echinococcus* eggs in soil and water, and livestock infection data from abattoirs complement human case detections by providing direct measures of environmental transmission pressure. Such integrated surveillance would also allow control programs to evaluate whether interventions (e.g., dog deworming) reduce environmental contamination, rather than relying solely on changes in human prevalence, which, given CE's long latency, may take years to shift.

Finally, the findings from the Bumthang district should be viewed as a starting point. Despite Bhutan's ecological and sociocultural similarities, transmission dynamics may differ across districts. Expanding community-based screening to other high-risk areas, guided by livestock infection data and canine surveillance, would provide a national-level evidence base needed to design and prioritize a coordinated One Health response. Bhutan's relatively small population, strong primary healthcare network, and existing One Health institutional structures offer a realistic foundation for incrementally building such a program.

Conclusion

This community-based ultrasound survey provides the first population-level estimate of the CE burden in Bhutan. The overall cluster-adjusted prevalence was 3.66% (including suspected CL cases), and the confirmed CE prevalence was 2.58% (95% CI: 1.93–3.44) when restricted to WHO-IWGE-staged cysts (CE1–CE5), with both estimates far exceeding hospital-based incidence figures. These findings highlight that CE is a substantial yet under-recognized public health problem in the Bumthang district. The co-existence of active and inactive cyst stages across age groups points to sustained community

transmission superimposed on long-standing infections, while the large proportion of previously undiagnosed cases confirms the inadequacy of passive facility-based surveillance for a chronic, often asymptomatic disease.

The risk factor findings have direct implications for the design of CE control. Intermittent dog contact was the only independently associated individual-level factor, while conventional predictors such as sex, occupation, and education were not, supporting the growing evidence that CE transmission in rural communities is related to wider environmental factors and is driven by shared exposure pathways rather than individual-level behaviors. In such settings, strategies targeting specific high-risk groups are unlikely to achieve sustained control of the disease.

These findings highlight the need for a One Health approach that addresses the interconnected roles of humans, animals, and the environment in CE transmission. Priorities should include the sustained and systematic deworming of both owned and free-roaming dogs, improved slaughter and offal management, strengthened environmental hygiene, and community-wide health education. Periodic community-based ultrasound screening linked to clinical referral pathways should complement hospital-based surveillance to capture the true burden and identify higher-priority areas, as indicated by the gewog-level variation observed in this study. Integrated environmental and canine surveillance would provide direct measures of transmission pressure and allow programs to evaluate intervention impact well before changes in human prevalence become apparent. In conclusion, effective CE control in Bhutan requires integrated, population-level strategies within the One Health framework. This cross-sectional study, by establishing a baseline of community prevalence and highlighting the importance of shared environmental risk, provides essential evidence to inform and guide One Health-oriented surveillance and control efforts in Bhutan.

Abbreviations

aOR: Adjusted odds ratio; CE: Cystic echinococcosis; CI: Confidence interval; CL: Cystic lesion; ICC: Intraclass correlation coefficient; IQR: Interquartile range; LRT: Likelihood ratio test; NTD: Neglected tropical disease; ODK: Open Data Kit; OR: Odds ratio; PHC: Primary Health Centre; PPS: Probability proportional to size; STROBE: Strengthening the Reporting of Observational Studies in Epidemiology; WHO: World Health Organization; WHO-IWGE: WHO Informal Working Group on Echinococcosis; WHO-PEN: WHO Package of Essential Noncommunicable Disease Interventions.

Declarations

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Data Availability Statement

All relevant data are within the manuscript and its Supporting Information files. The anonymized dataset is available from the corresponding author upon a reasonable request. In addition, the Ministry of Health owns the full dataset. Interested individuals may obtain the data upon request from the Chief Planning Officer, Policy and Planning Division, Ministry of Health, P.O. Box: 726, Kawangjangsa, Thimphu, Bhutan; Phone number- +975-2-328095, 321842.

Author Contributions

Conceptualization: PW and JZ. Data curation: PW. Formal analysis: PW and JH. Investigation: PW. Methodology: PW, JH, and JZ. Supervision: JH, JZ. Writing – original draft: PW. Writing – review and editing: PW, JH, and JZ.

Conflicts of Interest

The authors declare no conflicts of interest.

Human Ethics Declaration

All study procedures including ultrasound examination were performed in accordance with the Declaration of Helsinki. This study was approved by the Research Ethics Board for Health, Ministry of Health, Bhutan (approval number: Ref. No./PO/RL/2024.1.NNW) and by the Ethikkommission Nordwest- und Zentralschweiz (EKNZ), Switzerland (Study ID: AO_2024_00045).

Consent to Participate

Detailed information on study aims, procedures, risks and benefits, and voluntary nature of participation were explained to every participant in Dzongkha or the local dialect *(Bumthap)* and information sheet in Dzongkha and English were provided to those who could read. To those who could not read, the information sheet (Dzongkha version) was read aloud in the presence of a witness. Informed consent was obtained from all adult participants prior to study enrollment. For participants under 18 years of age, informed parental or legal guardians' consent and informed assent were obtained.

Clinical Trial Number

Clinical trial number: not applicable.

STROBE checklist

This study was reported in accordance with the STROBE guidelines for cross-sectional studies (S3 Checklist) as supplementary files.

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Figures

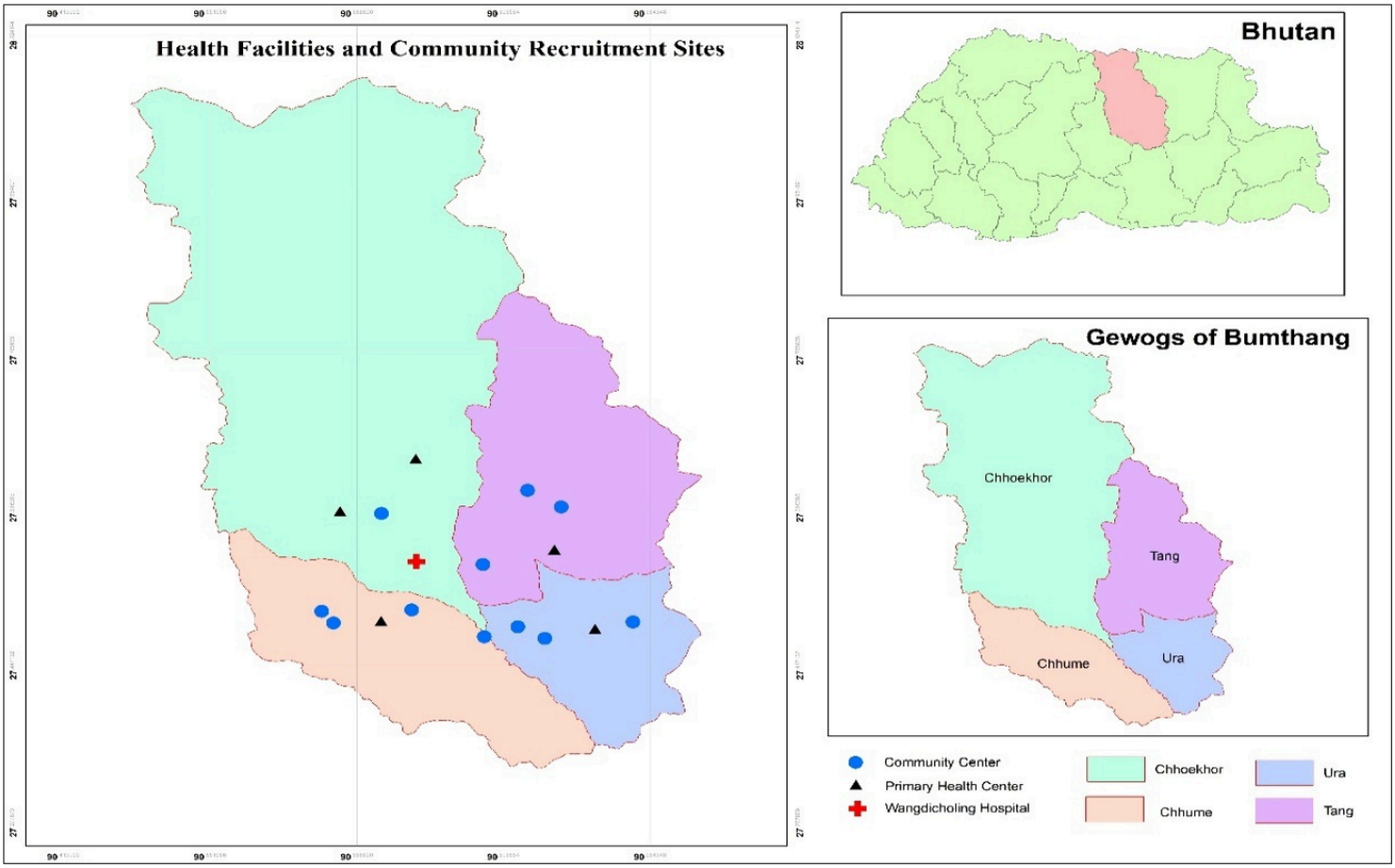


Figure 1

Study site showing the district hospital, PHCs and community centers for participants recruitment

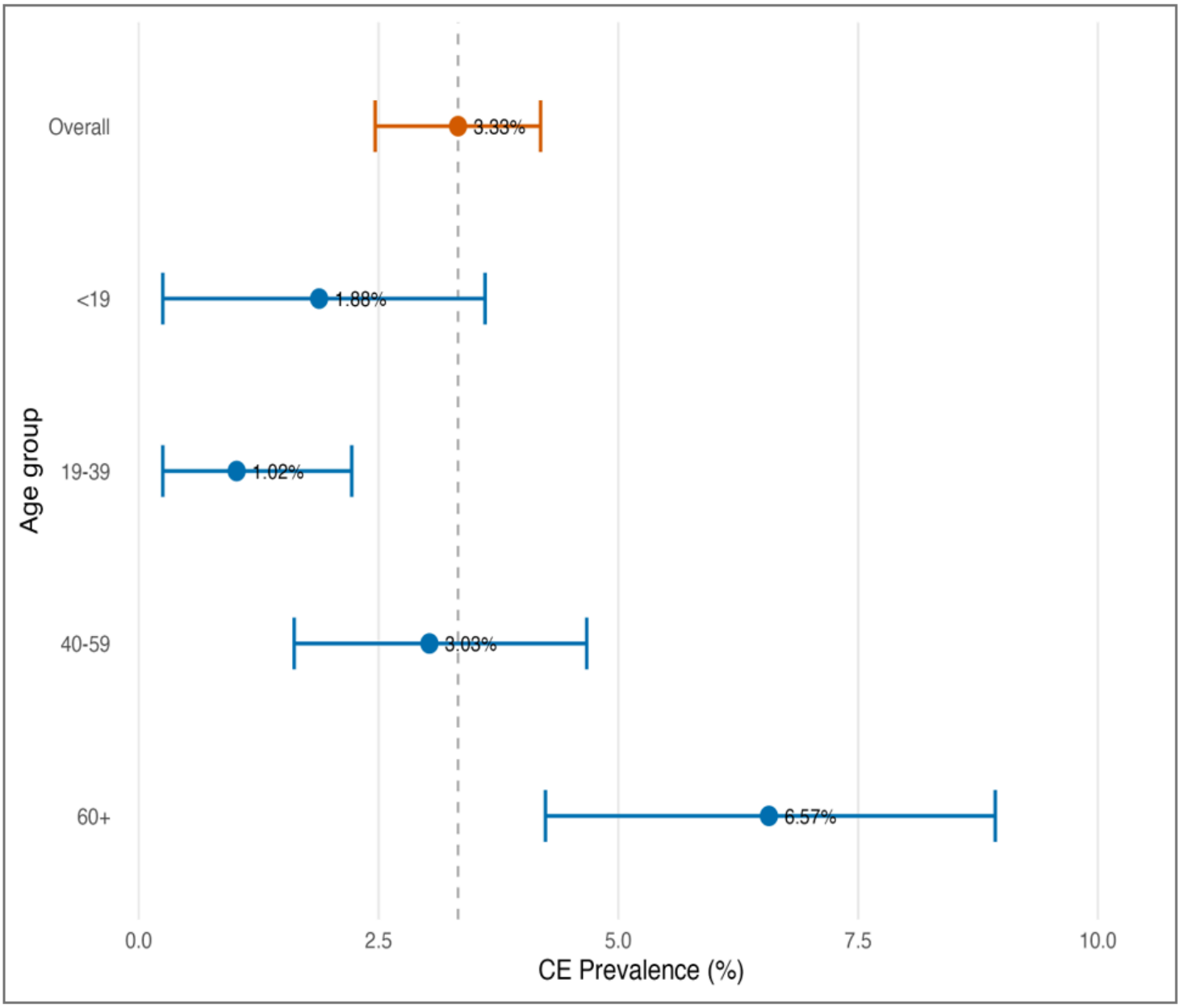


Figure 2

Forest plot showing the cluster-adjusted prevalence of CE (overall-reference dotted line, age-specific estimates using intercept-only logistic regression with cluster-robust SEs).

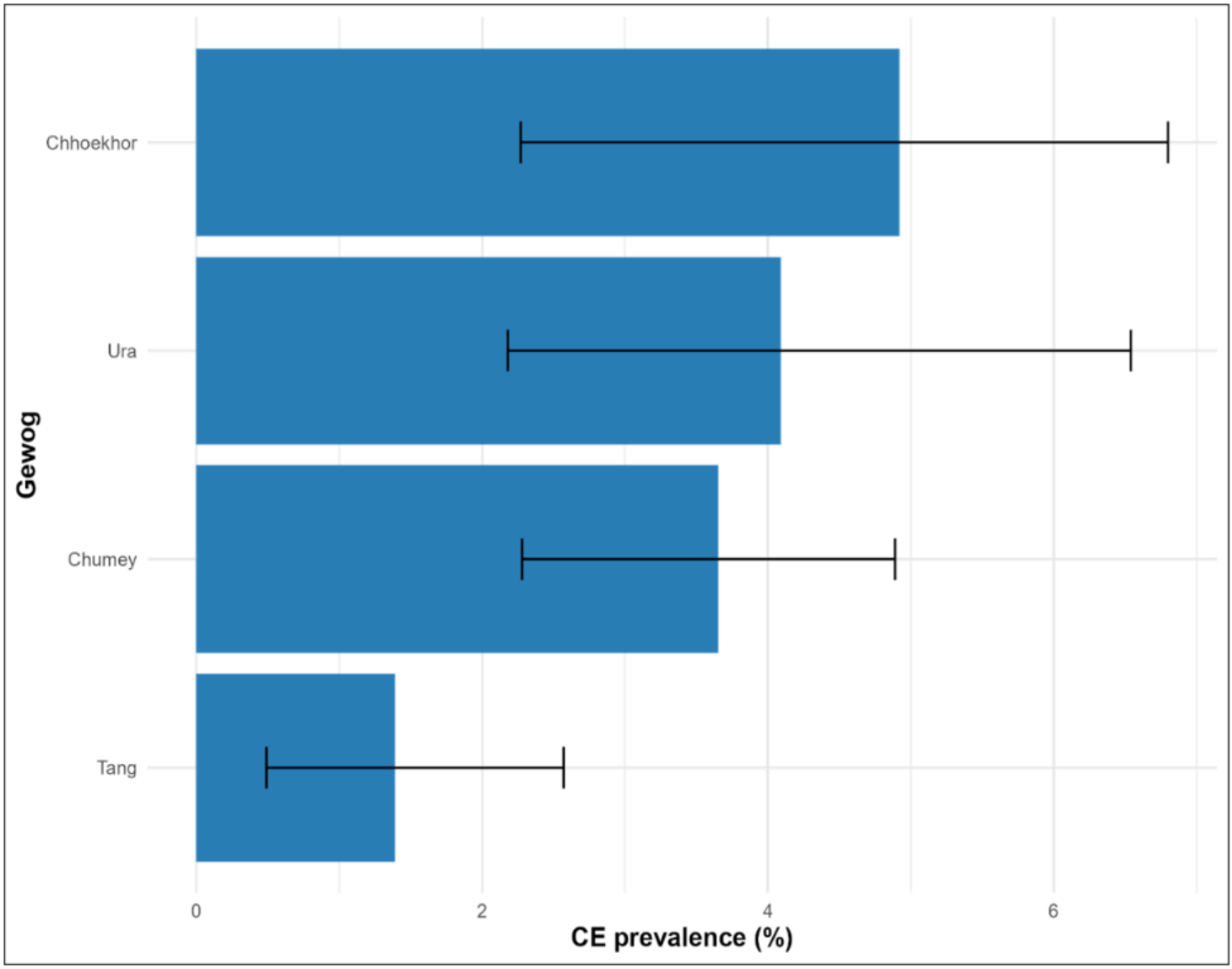


Figure 3

Cluster-adjusted gewog-wise prevalence of CE in Bumthang district.

Supplementary Files

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- SupplementaryMaterials.docx