Systematic Review

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Persistent febrile illnesses in Nepal: A systematic review

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***Background & objectives*: Although febrile illnesses are a frequent cause of consultation and hospitalization in low- and middle-income countries (LMICs), research has mainly focused on acute febrile illnesses (AFIs). In contrast, there are limited data on the causes of persistent febrile illnesses (PFIs) in LMIC. Lack of clarity on the differential diagnosis of PFIs in the rural tropics leads to the absence of diagnostic guidance tools.**

***Methods*: In this study, a review of the potential causes of persistent fever defined as fever of more than seven days was done in Nepal, with a focus on nine pathogen-specific conditions. The current knowledge on their burden, distribution and diagnosis was summarized.**

***Results*: Limited data were found on the incidence and public health burden of leptospirosis, murine typhus and brucellosis due to the absence of diagnostic tools outside reference laboratories and the overlap of signs and symptoms with other febrile conditions. The incidence of malaria and visceral leishmaniasis (VL) was found to be decreasing in Nepal, with some changes of the geographical areas at risk.**

***Interpretation & conclusions*: This review indicates a need for more research on the causes of PFIs in Nepal and in the region and for the development of clinical guidance tailored to current local epidemiology. Guidance tools should include specific clinical features (*e.g.* eschar), results of rapid diagnostic tests (*e.g.* malaria, VL), appropriate indications for more sophisticated tests (*e.g.* abdominal ultrasound, polymerase chain reaction) and recommendations for adequate use of empirical treatment.**

Key words Differential diagnosis - epidemiology - malaria - Nepal - persistent febrile illnesses - visceral leishmaniasis

Febrile illnesses, a frequent cause of consultation and hospitalization in low- and middle-income countries (LMICs), can arise from diverse infectious agents, including viruses, bacteria and parasites[1,2]. Research has mainly focused on elucidating the causes of acute febrile illnesses (AFIs) as shown by landmark studies in Africa and Asia that only included patients with less than one week of fever[3-5]. In contrast, there are scarce data on the causes of persistent febrile illnesses (PFIs) in LMICs[1]. The concept and definition of fever of unknown origin (FUO) was developed >50 yr ago[6], and clinical management has evolved since but still requires a range of investigations[2] that are not feasible in LMICs outside a few tertiary care centres. Lack of

knowledge on the differential diagnosis of PFIs in the rural tropics results in a lack of diagnostic guidance, apart from some diagnostic algorithms that focus on a single disease, such as visceral leishmaniasis (VL)[7].

Nepal is bordered by China and India, spanning 147,181 km^2 with a population of 26,494,504. Ecologically, it is divided into the plains (called Terai), hills and mountain regions based on differences in elevation[8]. Managing PFIs in Nepal is challenging, mainly due to knowledge gaps, lack of awareness about the differential diagnosis and insufficient diagnostic facilities. While several studies have been conducted on determining the causes of AFIs in Nepal, none to our knowledge has focussed on PFIs[9-15].

This study was aimed to review potential causes of persistent fever defined as fever of seven days or more in Nepal. Our review focuses on a selected group of nine pathogen-specific conditions that are potentially severe and treatable and persist if not treated appropriately. For each selected condition, current knowledge on its burden, distribution and diagnosis in Nepal is summarized.

Material &Methods

Eligibility criteria: The literature was searched for studies about nine target infections (amoebic liver abscess, brucellosis, enteric fever, leptospirosis, malaria, melioidosis, rickettsial diseases, tuberculosis, visceral leishmaniasis) affecting human subjects in Nepal. Non-pathogen-specific conditions such as pneumonia, cholecystitis, pyelonephritis, pelvic inflammatory disease or non-treatable illnesses, such as infectious mononucleosis due to Epstein-Barr or cytomegalo virus were excluded. Further, Knowledge, Attitude and Practice (KAP) - surveys, entomological surveys, mathematical modelling studies, genetic research and economic impact studies were also excluded. Studies focusing on HIV-infected people only were not included. All study designs and reporting formats including case reports were eligible, except for reviews and opinion articles. The PRISMA flow diagram is presented in Fig. 1.

Information sources: We searched PubMed, Web of Science, Scopus and CAB Direct (Global Health) up to April 2017. The search strategy combined 'Nepal' and the respective 'diseases'. Google Scholar was also searched to look for unindexed/grey literature. The first 1000 results from Google Scholar were retrieved, using Harzing's Publish or Perish software 5.27.0.6259 (*https://harzing.com/resources/publish-or-perish*)

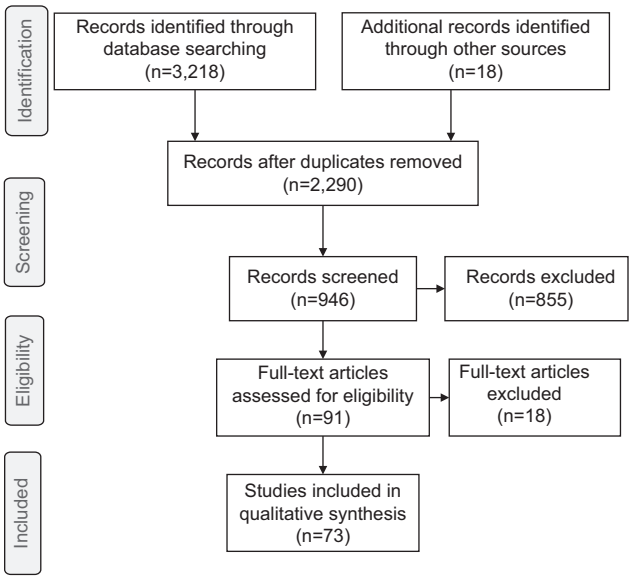

Fig. 1. PRISMA flow diagram of the selection of literature for the review of persistent febrile illnesses in Nepal.

and the titles were screened for the most relevant. The reporting on ProMED-SoAs (*https://www.promedmail.org/*) was included to track outbreaks of febrile diseases in the country. Country data were also collected from the annual health reports of Nepal. Finally, the reference lists of the included papers were manually checked for additional records that were not retrieved by automatic database searches. There were no restrictions on language and publication date.

Study selection: Based on eligibility criteria described above, the titles and abstracts of all articles identified by the search strategy were screened and a set of full-text papers were read to select the studies for this review. After removal of duplicates, references were compiled per disease of interest and added to EndNote Web.

Data collection and synthesis: For each of the target conditions, information was extracted about frequency and diagnosis in Nepal. Since our objective was to give a broad overview of the available literature, no restrictions were imposed on study design. As a result, the included studies were diverse, and it was not possible to formally and systematically assess the risk of bias in individual studies. The extracted information was synthesized using narrative description. The Table shows the frequency of target conditions among febrile patients in Nepal giving overviews of available information per target condition[9-27] and Figs 2 and 3 shows numbers of reported cases of malaria and visceral leishmaniasis over time.

Table. Studies conducted in Nepal reporting the aetiologies of fever targeted

Author	Type of study	Laboratory tests used	Frequency of target diseases	
Murdoch *et al*, 2004[9]	Prospective follow up study, inpatient and outpatient n=876	Blood culture and RDTs	Enteric fever	13%
			Rickettsial diseases	11%
			Leptospirosis	4%
Sharma *et al*, 2006[10]	Retrospective review of medical and laboratory records, inpatient and outpatient n=1774	Blood culture	Enteric fever	6.9%
Blacksell *et al*, 2007[11]	Prospective follow up study, inpatient and outpatient n=103	Indirect immunofluorescence assay (scrub and murine typhus), IgM capture ELISA (leptospirosis), blood culture	Murine typhus	27%
			Scrub typhus	23%
			Enteric fever	22%
			Leptospirosis	10%
Dhungana *et al*, 2012[12]	Retrospective review of medical and laboratory records in patients n=898	Routine laboratory testing and clinical diagnosis	Culture negative enteric fever	12.8%
			Culture positive enteric fever	7.3%
			Pulmonary TB	7.3%
			Brucellosis	0.8%
			Leptospirosis	0.5%
			Malaria	0.4%
			Visceral leishmaniasis	0.3%
			Murine typhus	2%
Pradhan *et al*, 2012[13]	Prospective cross-sectional, outpatients n=1084	Blood culture PCR (*R. typhi*)	Enteric fever	13%
			Positive PCR for *Rickettsia. typhi*	2%
Bhatta *et al*, 2013[14]	Retrospective review of medical and laboratory records, inpatient and outpatient n=4145	Blood culture	Enteric fever	12%
Shankar *et al*, 2014[15]	Prospective cross-sectional, inpatient and outpatient n=2873	Routine laboratory testing and clinical diagnosis	Enteric fever	10%
			Malaria	3%
Zimmerman *et al*, 2008[16]	Prospective cross-sectional, inpatient and outpatient n=876	PCR	Positive PCR for *R. typhi*	7%
Myint *et al*, 2010[17]	Prospective follow up, army personnel who volunteered for hepatitis E vaccine trial n=271	IgM ELISA followed by MAT, which was confirmed by four-fold rise	Leptospirosis	6.1/1000
Kandel *et al*, 2012[18]	Retrospective cross-sectional, serum samples from acute encephalitis syndrome surveillance n=974	Latex agglutination assay	Leptospirosis	37% (IgG); 43% (IgM)

Contd..

Author	Type of study	Laboratory tests used	Frequency of target diseases	
Nepal *et al*, 2013[19]	Prospective cross-sectional, hospital-based collection n=1226	Panbio IgM ELISA (Inverness Medical Innovations, Australia) no paired serum samples	Leptospirosis	4.8% (IgM)
Dahal *et al*, 2016[20]	Prospective cross-sectional, laboratory based n=178	Panbio IgM ELISA (Inverness Medical Innovations, Australia) no paired serum samples	Leptospirosis	28.7% (IgM)
Nepal *et al*, 2016[21]	Prospective cross-sectional, inpatient and outpatient n=365	Serum agglutination test ≥1:160	Brucellosis	18.1%
Upadhyaya *et al*, 2016[22]	Prospective cross-sectional, laboratory based n=434	Detect TM IgM ELISA (InBios International, Seattle, WA, USA)	Sero-prevalence IgM to *Orientia tsutsugamushi*	40.3%
Bhattachan *et al*, 2016[23]	Prospective, cross-sectional on randomly selected samples that were negative for Japanese encephalitis n=993	Latex agglutination test	Leptospirosis	35.5%
Regmi *et al*, 2017[24]	Prospective cross-sectional, outpatients n=114	Panbio IgM ELISA (Inverness Medical Innovations, Australia) No paired serum samples	Leptospirosis	20.8% (IgM)
Brown *et al*, 1981[25]	Prospective cross-sectional, healthy army recruits and blood donors n=188	Micro-immunofluorescence on unpaired serum samples	Seroprevalence *O. tsutsugamushi*	10%
Joshi 1984[26]	Prospective cross-sectional, inpatient and outpatient n=2117	Serum agglutination test and 2-mercapto-ethanol tests	Brucellosis	2.7%
Rai *et al*, 2000[27]	Prospective cross-sectional, outpatients n=200	One-point MCA method 1:300	Leptospirosis	32%

MCA, microcapsule agglutination; MAT, microscopic agglutination; RDTs, rapid diagnostic tests; TB, tuberculosis; PCR, polymerase chain reaction

Results

A total of 3218 articles were found through the systematic search of standard literature databases and retrieved 18 additional records, including annual health reports. After removing the duplicates, screening the titles and abstracts and reading a set of full-text papers, 73 studies were included in this review (Fig. 1).

Amoebic liver abscess: The incidence of amoebic liver abscess caused by *Entamoeba histolytica*[2] in Nepal is unknown. There has been documentation of 27 cases in a hospital near Kathmandu from 1999 to 2003[28] and 24 cases among 36 patients with liver abscess from Eastern Nepal from 1995 to 1998[29]. A prospective observational study that recorded and analyzed clinical presentation, diagnosis and treatment of patients with liver abscess in a teaching hospital found that 61 per cent of the liver abscesses were caused by *E. histolytica*[30].

Brucellosis: Brucellosis is caused by a Gram-negative bacteria *Brucella* sp. and is transmitted from animals to humans by ingestion, direct contact or inhalation[2]. As a large number of young people are involved in livestock raising in Nepal, the burden of brucellosis in animals

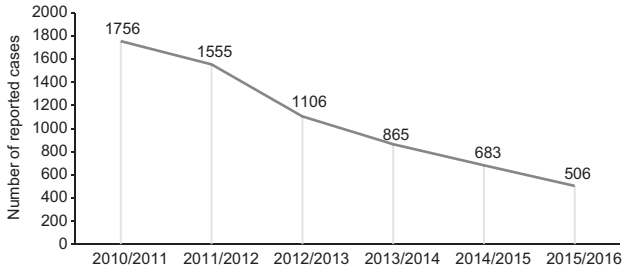

Fig. 2. Indigenous cases of malaria in Nepal based on reported cases. *Source*: Refs 38, 39, 49, 75-77.

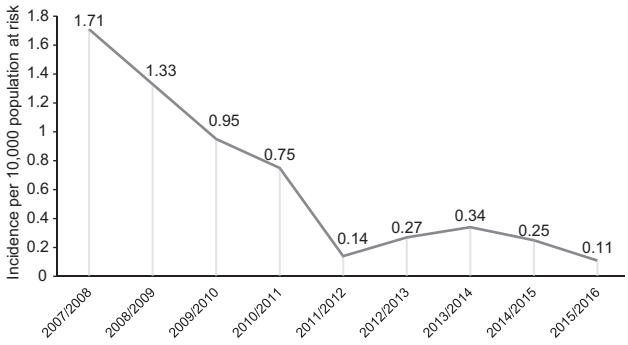

Fig. 3. Incidence of visceral leishmaniasis per 10,000 population at risk in Nepal based on reported cases. *Source*: Refs 38, 39, 75-79.

and humans needs to be considered[31], but the data are limited. The Department of Health Services reported 112 human cases (diagnosed based on serology) from 1997 to 2002[31]. In a sero-epidemiological survey conducted in 1983, the seroprevalence of brucellosis in Kathmandu Valley was reported to be six per cent[31] compared to a recent study that found a seroprevalence of 18.1 per cent (Table)[21]. In Nepal, only serological tests are currently available in a limited number of reference laboratories.

Enteric fever: Enteric fever, caused primarily by *Salmonella enterica* serovar Typhi and Paratyphi, is a major public health problem in Nepal[9-15,32-37]. Although its exact burden is not known, Kathmandu has been referred to as 'enteric fever capital of the world'[35]. Furthermore, suspected enteric fever is one of the top ten reasons for outpatient consultation in Nepal[38]. The major determinants for this high occurrence are reported to be poverty, poor sanitation and inadequate facilities for safe drinking water[36]. According to the Ministry of Health and Population, 8926 people were hospitalized due to enteric fever in Nepal in 2014-2015[38], with an estimated annual incidence of around 100 per 100,000 population[38]. In 2015-2016 there was an outbreak in Kathmandu with 67 cases[39]. A study that reviewed blood cultures performed between 1993 and 2003 in a tertiary hospital in Kathmandu yielded *S.* Typhi or *S.* Paratyphi in approximately 75 per cent of all positive blood cultures[32]. Similarly, another study recorded 9901 cases of blood culture-proven enteric fever in Kathmandu district over a period of five years[33]. Several studies conducted among febrile patients in Nepal showed a high prevalence of enteric fever ranging from 7 to 22 per cent (Table)[9-15].

Although blood culture is the reference standard to diagnose enteric fever, the Widal test is still commonly used in Nepal as cultures are not available in most healthcare facilities[40]. The Widal has limited diagnostic performance that is further impaired by the lack of consensus on the test's interpretation[41]. Simpler and high performance diagnostic tools are therefore needed. As an alternative for classical blood culture, a simple phase-change incubator, not requiring electricity or laboratory infrastructure, was assessed in Nepal, showing an overall per cent agreement between the experimental and the traditional incubator of 94.4 per cent[42]. A recent Cochrane review evaluated 37 studies that assessed the diagnostic accuracy of 16 rapid diagnostic tests (RDTs) for enteric fever. The three most often studied RDTs, *i.e.* TUBEX (IDL Biotech AB, Bromma, Sweden), Typhi dot (Malaysian Biodiagnostic Research, Bangi, Malaysia) and Test-It Typhoid (Lifeassay Diagnostics, Cape Town, South Africa), showed moderate diagnostic performance with sensitivity estimates ranging from 69 to 84 per cent and specificity estimates from 79 to 90 per cent[43].

In Nepal, there is a notable increase in resistance to nalidixic acid, leading to resistance to commonly used medicines in enteric fever[44]. However, surprisingly, resistance to ceftriaxone remained low, and there was decreasing resistance to chloramphenicol and co-trimoxazole, presumably associated with declining use of these antibiotics, resulting in the restoration of these agents as therapeutic options[45].

In 1987, the efficacy of a Vi polysaccharide vaccine (Vi-PS) was assessed in a large double-blind randomized trial in the age group 5-44 yr olds in Nepal; this trial showed a protective efficacy of 72 per cent at 17 months of follow up[34]. However, this vaccine has not been implemented in routine practice as it is very expensive and unaffordable for Nepal[33]. A large school-based immunization programme with Vi-PS vaccine by a non-profit organization was conducted in 2001to assess the vaccine safety for a school-based

immunization system[46]. It has been suggested that the cost of the vaccine and use of easily accessible over-the-counter antibiotics weaken the attempt for a nation-wide vaccination campaign[33,47]. There are also several water and sanitation programmes run by various organizations, that aim to decrease the incidence of enteric fever and other faeco-oral transmitted organisms in the country[48], like the SAFE-WASH project supported by the United States Agency for International Development (USAID) and programmes by the Gurkha Welfare Trust, along with the Department for International Development (DFID).

Leptospirosis: Leptospirosis is caused by spirochetes of the genus *Leptospira*, transmitted through skin contacts with water or moist soil contaminated with urine of infected rats or other mammals. Although leptospirosis is identified as a priority zoonotic disease in Nepal with epidemic potential since 2012/2013[49], there is no epidemiological surveillance programme for leptospirosis and national figures are lacking. None of the outbreaks of febrile illnesses in Nepal has been attributed to leptospirosis[9,18], although ideal conditions exist for the transmission of this pathogen in the country[50]. There are a few serological studies carried out in Nepal showing the presence of antibodies against major *Leptospira* spp. prevalent in Asia[9,11,12,17-20,23,24,27] and underlining that the infection is largely underreported[9]. An annual incidence of 6.1 cases per 1000 population was found in a cohort of healthy volunteers[17]. Among febrile patients, seroprevalence reported in various studies ranged from 0.5 to 37 per cent (Table)[9,11,12,17-20,23,24,27].

Due to the lack of laboratory techniques to accurately diagnose and the non-specific presentation of the disease, leptospirosis is under- or over-reported hence explaining the wide range of seroprevalence in the studies reported in Table[9,11,12,17-20,23,24,27]. The microscopic agglutination test (MAT) is the reference serological test, but it is cumbersome, expensive and not readily available in Nepal[19,20]. The lack of specificity of IgM ELISA has probably led to an overestimation of the prevalence of leptospirosis among febrile patients in several studies conducted in Nepal (Table). RDTs have been developed showing up to 85 per cent sensitivity at the end of the first week of illness with a high specificity of 94 per cent[51].

Malaria: Malaria is a life-threatening haemoprotozoan infection caused by one of the five *Plasmodium* species, transmitted to humans by the bite of *Anopheles* mosquitoes[2]. According to the national microstratification report published in 2013, 13 million Nepalese people (47.9%) live in malaria-endemic areas, of whom about 1 million (3.6%) live in high-risk, 2.7 million (9.8%) in moderate-risk and 9.4 million (34.5%) in low-risk areas[39]. Over the last decade, a drastic decrease by more than 84 per cent in the incidence of malaria was observed in Nepal[52], as shown in Fig. 2. Factors that may have contributed to the decline in the number of malaria cases are the introduction of artemisinin combination therapy for the treatment of uncomplicated *Plasmodium falciparum* cases in 2004, the distribution of long-lasting insecticidal nets in high-risk priority districts since 2005, indoor residual spraying in high-endemic foci and free health service delivery[52-54].

The diagnosis of malaria is confirmed by microscopic examination of thin and thick blood smear or specific antigen detection by RDTs. The OptiMAL dipstick (Flow Inc., Portland, OR) has been proved to be useful in diagnosing *P. falciparum* and *P. vivax* infections with high sensitivity (96%) and specificity (100%)[55] and is recommended as a diagnostic tool by the Epidemiology and Disease Control Division (EDCD) of Ministry of Health and Population of Nepal for in-field detection or when microscopy is not available[48].

Nepal has adopted a long-term malaria elimination strategy 2011-2026 with the vision of a malaria-free Nepal by 2026[52]. The recent drop in reported cases may be jeopardized by climate change and the increasing number of dams[56]. Moreover, malaria transmission has been observed in areas that were previously considered non-endemic and where no vector control interventions were implemented so far[57]. Other potential threats to malaria elimination in Nepal include emerging drug resistance in the parasite, increasing insecticide resistance of *Anopheles* mosquitoesand large numbers of imported cases from other countries[52].

Melioidosis: Melioidosis is an infection caused by *Burkholderia pseudomallei* with a wide range of disease manifestations ranging from localized abscesses to fulminant septic shock. It is widely endemic in South-east Asia and Northern Australia and is emerging in the two neighbouring countries of Nepal - India and China[58]. The Terai part of Nepal has all the characteristics of a high-risk area with a tropical climate, farming as the predominant occupation and a growing diabetic population in the region. There

has been no report of indigenous transmission of melioidosis in Nepal to date, but the diagnosis could have been overlooked due to the lack of available diagnostic tools (culture of blood or other biological fluid). The only report of melioidosis in Nepal involved a person returning from Malaysia after a one-year stay[59].

Rickettsial diseases: Rickettsial infections are arthropod-borne infections caused by various species categorized into the typhus group and the spotted fever group. Scrub typhus, caused by *Orientia tsutsugamushi* and transmitted by chigger mites, and murine typhus, caused by *Rickettsia typhi* and transmitted by rat fleas, are both endemic in Nepal[9,11,16]. After the 2015 earthquake, there was a rapid increase in the cases of scrub typhus reported from July to November 2015 in 37 of the 75 districts of Nepal[60], with outbreaks in nine districts[39]. The highest antibody titres (IgM) against *O. tsutsugamushi* were found in serum samples collected from Dhading, Kailali, and Kanchanpur, followed by Ramechhap, Khotang and Rautahat districts[22]. Studies on rickettsial diseases in Nepal are listed inTable[9,11-13,16,22,25].

The EDCD of Nepal advises that patients with undifferentiated febrile illness of five days or more with or without eschar (or less than five-day illness with eschar) should be suspected of having a rickettsial infection[48].

Tuberculosis: Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* which primarily affects the lungs, causing pulmonary tuberculosis (PTB), but can affect other organs[2]. In Nepal, about 45 per cent of the total population is infected with *M. tuberculosis* (both latent and active infection)[61]. In 2016, 45,000 cases of TB were registered in Nepal with an estimated annual incidence of 154/100,000. The multi drug-resistant TB among the PTB were 2.2 and15 per cent among new and previously treated cases, respectively[62].

Molecular diagnosis by the GeneXpert MTB/RIF assay for the detection of *M. tuberculosis* in the sputum was shown to be more sensitive than microscopy after Ziehl-Neelsen or Auramine staining[63,64]. However, the GeneXpert is not available in all centres. There are around 30 GeneXpert machines deployed in Nepal at selected centers[65], with 23,818 tests done in 2016. A study compared a transport reagent OMNI gene® SPUTUM against Nepal's standard of care transport system. The OMNI does not require a cold chain and proved useful for long-term transport (up to 2-13 days) of sputum sample for smear and culture[66]. There are currently only four centres in Nepal that are equipped to perform sputum culture for TB[65].

Visceral leishmaniasis (VL): In Nepal, VL is a public health problem in 12 out of 75 districts, all bordering India. The incidence rate of VL at national as well as district level is now less than 1/10,000 population per year. The incidence ranges from 0.01 to 0.47 (mean: 0.25) per 10,000 in the 12 endemic districts (excluding imported cases from India)[38]. Although its incidence in Nepal has decreased (Fig. 3), VL is now increasingly reported from districts previously classified as non-endemic districts[67]. Autochthonous VL cases have been reported from new areas mostly in hill and mountain regions of Nepal[68-72]. Evidence of local transmission of *Leishmania donovani* in some hilly districts of Nepal was seen as (*i*) most VL patients did not report travels to historical endemic districts or to India, (*ii*) 9.6 per cent of asymptomatic residents showed signs of previous or current *Leishmania* infection (as shown by positive serology or PCR), and (*iii*) *Phlebotomus argentipes* sand flies, the vector of VL in this region were present in the hilly districts[72].

RDTs are commonly used for confirming the diagnosis of VL in clinical suspect patients. According to a systematic review, the sensitivity of rK39 antigen-based RDTs in South Asia was 97 per cent and the specificity was 90.2 per cent[73]. Other serological tests such as the direct agglutination test showed excellent diagnostic performance but were only available in a few reference laboratories. The main strategies are early (decentralized) diagnosis, effective treatment and vector control. Nepal has reached the elimination target in 2014 and is now in the consolidation phase (2015-onwards)[39].

Discussion

This review summarized epidemiological data, currently implemented or recommended for nine pathogen-specific conditions causing PFIs in Nepal. The incidence of conditions that are diagnosable, reportable and targeted by specific control programmes in the Nepalese population, such as tuberculosis, malaria and VL, are known at country and district levels[38,49,74-77]. However, limited data exist on the incidence and public health burden of the other conditions. For example, leptospirosis, murine typhus and brucellosis are likely to be overlooked due to the absence of diagnosis tools outside reference laboratories and the overlap of signs and symptoms with other febrile conditions[78]. More epidemiological and clinical studies are needed to define the true burden of leptospirosis, murine typhus

and human brucellosis in Nepal. In contrast, in the absence of blood culture facilities, enteric fever is over-diagnosed at primary and secondary healthcare levels, due to the lack of specificity of clinical features and of the widely used Widal test[79]. Other conditions such as tick-borne relapsing fever or melioidosis have not been reported in Nepal; however, as no specific investigations are done, this absence of reporting does not exclude their sporadic presence.

What is not known for any of these conditions is their relative contribution to explain persistent fever. A limited number of studies that investigated the causes of febrile illnesses in Nepal have focused on patients with acute fever or fever of any duration[9-15]. We did not find studies that focused on PFIs, except studies that evaluated the diagnostic performance of new tools for VL in patients with clinical suspicion, *i.e*. fever more than two weeks and splenomegaly[80]. In addition, the vast majority of studies that investigated causes of fever were conducted in teaching hospitals located in the Kathmandu area, which might not be representative of the rest of the country[9-13,15].

There is a need for more research on the causes of PFIs in Nepal and in the region. The spectrum of causal conditions will partly differ from the conditions causing acute fever, excluding most viral infections (*e.g*. dengue, upper respiratory tract infections). Moreover, as the incidence of malaria and VL is decreasing, other less-known diagnoses, such as scrub typhus and leptospirosis, are emerging to become more prominent. In addition, the performance of several diagnostic tools for PFI is still inadequate, with a suboptimal sensitivity of pathogen detection in the blood by culture (*e.g*. enteric fever) or PCR (*e.g*. scrub typhus, leptospirosis) and suboptimal specificity of serological-based tests, as shown for leptospirosis.

There is also a need for continued professional training to enhance the understanding of PFI by health workers. The difficulty in establishing the cause of febrile illnesses has resulted in omission or delays in treatment, irrational prescriptions with polypharmacy, increasing cost and development of drug resistance. Guidance tools for the management of PFIs should be developed that take into account specific clinical features (*e.g*. eschar), results of RDTs (*e.g*. malaria, VL) and appropriate use of more sophisticated tests (*e.g*. GeneXpert). Recommendations for appropriate use of empirical treatment are also required. The clinical guidance tool should be tailored to the local epidemiology. Several studies have been conducted by the Neglected Infectious diseases DIAGnosis (NIDIAG) research consortium that aimed to determine the causes of persistent fever and develop evidence-based guidance tools in Nepal and other locations in Asia and Africa[81], but more research on this topic is warranted.

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