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Brucellosis in Nepal - A Potential Threat to Public Health Professionals

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ABSTRACT: Brucellosis is a prominent zoonotic disease affecting humans and animals which with the lack of proper diagnosis and treatment remains dangerous in third world countries like Nepal. Currently, Brucellosis poses a public health concern, whose incidences among entire herds of animals can present substantial economic and health burdens for herders and health professionals. Additionally, factors such as close contact with animals, poor animal husbandry, and unhygienic feeding habits can exacerbate the spread of Brucella and related zoonotic agents. In Nepal, serious cases of bovine and even human brucellosis have been reported, although the topic is yet to be extensively reviewed. This paper evaluates the literatures on human and animal brucellosis in Nepal and other countries, with an emphasis on the impact of Brucella outbreaks on public health professionals. Herein, we summarize the current status of the disease, the mechanism of infection, pathogenesis, zoonotic potential, diagnostic advances, treatment regimens, and the preventive measures that can be adopted in managing human brucellosis in under-developed countries such as Nepal.

KEYWORDS: Brucellosis; zoonotic status; Public health professionals; Nepal.

Introduction

Brucellosis is a major abortifacient zoonotic disease of livestock with worldwide distribution [1, 2]. *Brucella abortus* that cause bovine brucellosis along two other *Brucella* species namely *B. melitensis* and *B. suis*, is of veterinarian importance and are highly pathogenic that cause miscarriage in second half of pregnancy, infertility in females and sterility in males their natural hosts [2-4]. Establishment of the carrier state in a large proportion of animals can lead to a significant reduction in milk yield which together with losses through abortion or early calf death due to *B. abortus* infection is a huge economic constraint for farmers [5, 6]. In regions where disease surveillance and control measures are not instigated, long-term chronic infections are often associated with carpal hygromas and infertility [1, 7]. Disease presentations in bulls include orchitis, epididymitis and seminal vesiculitis [4, 6, 8]. Brucellosis is a major public and animal health problem in areas with intensive mixed types of farming and where owners cohabit with their animals during night [2, 9, 10]. The mixed, migratory and free roaming nature of the livestock herding makes it unpractical to separate the suspected and healthy animals thus ensuring a favorable environment for *Brucella* transmission. There is system of mixed type of animal farming which provides conducive environment for the transmission of brucellosis.

In Nepal, brucellosis is a serious public health threat posed by endemic bovine and caprine brucellosis. Recent cases of human brucellosis in veterinary and para-veterinary students have been alarmingly serious. A systematic and large scale brucellosis control program targeting the eradication of the disease has not been adopted in Nepal. Financial constraints, inadequate laboratory facilities, lack of compliance by the farmers and religious taboo have been major hurdles that impede the success of *Brucella* eradication in Nepal. Presently, control measures have been implemented in only few commercial farms. However, lack of precise information on the distribution, public health and economic impact of brucellosis is attributed to disease of low priority by policy makers and limited resources allocated for the control of this disease.

Though it has been widely reported in various species of animals and humans, there is a dearth of literature addressing this disease in the very context of Nepal. Thus, the main purpose of this article is to review the extensive literatures available so far and suggests some guidelines that fit the context of Nepal.

Transmission

Chronically infected cattle may shed the organism via milk and reproductive tract discharges [11-13], and can also vertically transmit infection to subsequently born calves, thereby maintaining disease transmission [1,14]. Aborted fetuses from infected animals contain huge numbers of infectious organisms and if not properly disposed form a major source of contamination [15]. The pathogen is highly

contagious and is easily spread by licking of infected animals and abortion materials [16, 17], and abortion materials, discharges and waste of infected animals may contaminate stables, meadows, food supplies and water sources [18, 19]. Direct contact with infected animals and consumption of contaminated dairy products may cause infection in human beings [20, 21]. Human to human transmission is relatively uncommon [22]; however, it had been reported to develop after blood marrow transplantation [23, 24], sexual intercourse [22], and also following blood transfusions [25]. Animals in Nepal are often housed in unhygienic sheds with poor management systems and also in close association (some sharing the same roof) with each other. This presents significant risk factors for the contraction of brucellosis in humans. Similarly the consumption of raw milk, liver, spleen, udder, kidney and testis and handling of dung, which is widely prevalent in Nepal, may also, serve as source of hazard. Practices like Yak's raw blood consumption on faestive scale also pose a threat yet to be studied.

Pathogenesis and Immune Response

The ability of the pathogen to survive and replicate within different host cells explains its pathogenicity [26, 27]. The pathogenesis depends upon various factors such as the species, size of innoculum, modes of transmission and immune status of host [28]. Extensive replication in placental trophoblasts is associated with abortion [1, 28, 29], and persistence in macrophages and other cell types leads to chronic infections [30-32]. Protective immunity to the host is conferred by T-cell mediated macrophage activation by antigenic protein of Brucella and production of corresponding antibody along with involvement of various players such as Tumor Necrosis factor (TNF), interferons and complements. Following infection, Ig M titer increases initially followed by Ig G titer. Thus, the apperance of IgM indicates immune response against brucellosis and IgG correspondingly indicates chronic infection or relapsed form [33].

Causative Agent

Brucellosis is caused by infection with gram-negative bacilli of the genus *Brucella*. The genus encompasses nine recognized species including three species that are of economic importance [1, 34] and of which *B. melitensis* predominantly infects sheep and goat, *B. abortus* infects cattle, and *B. suis* infects swine [1]. These species may also infect camaloids, jacks and a variety of wildlife species. *B. melitensis, B. abortus*, and *B. suis* can be further sub-divided in biovars, and further sub-species differentiation is possible using molecular tools.

Global Situation

Although some of the European and Asian countries have been declared free of Brucella, it is still widely prevalent [32, 35]. At present global burden of brucellosis is found to vary from 1 to 200 per 10 millions per year [35, 36]. According to the available data, incidence of human brucellosis is increasing in Eastern Mediterranean countries [37]. Moreover, it is endemic disease in Asian countries, such as Sri Lanka [38], India [39], China [40], Pakistan [41], Mongolia [42], Nepal [43-45]. Despite some efforts to eradicate brucella from animals, most have been terminated due to lack of sufficient funds [2, 46]. Prevalence of both animal and human brucellosis is significant in rural nomadic communities of many countries, including Nepal. Nonetheless, the true incidence of human as well as animal brucellosis is still unknown for developing countries (including Nepal). This lower incidence of brucellosis in endemic areas, such as Nepal, denotes either the absence or low level of disease surveillance and monitoring.

Nepalese Situation

Regarding the literatures, there is a small number of published papers addresing the brucellosis. Most of the papers focused on bovine brucellosis with sparse information available to address brucellosis in humans and small ruminants. The available literatures indicate that bovine brucellosis in endemic in Nepal and *B. abortus* is the most predominant followed by *B. suis. B. abortus* is predominant in cattle accounting a substantial portion of bovine abortion in the country. Unfortunately, nothing has been done to identify the species of *Brucella.*

Though the brucellosis is reported to be endemic in Nepal, neither the distribution nor the economic and public health impact of this disease is well characterized [45,47]. This warrants a strong recommendation of better and economically feasible control option. It is equally important to improve the present control programmes. A case where an university livestock farm got infected with brucellosis because of an introduction of infected bulls

imported from India is still fresh in our memories.

Brucellosis in Animals

The presence of brucellosis in Nepal was first established in 1977 by Pyakurel and Mishra [48]. Since then it has been reported from various parts of Nepal [49-54] [Table1]. Pyakurel and Mishra [48] studied the sero-epidemiology of brucellosis in animals within Nepal and reported the highest prevalence [22.64%] in buffaloes from Pokhara. Joshi [49] reported a prevalence of 6.08% in humans, 8.7% in cattle, and 3.64% in sheep and goats. Department of Health Service (DHS) Nepal has reported that about 2-3% of cattles in Nepal are sero-positive for brucellosis. The published report, including the recent findings, clearly indicates that human and animal brucellosis is quite common in Nepal [45,48]. Brucellosis is found to vary between different species of animals [17.6% in Yak, Nak and Chauri, 10% in dogs, 8.7% in cattle and buffalo, 3.64% in sheep and goat, 7.18% in pigs] and also in terms of regions [1.49% to 5.36%] with highest number of cases found in Solukhumbu [5.36%] and the lowest in Pokhara [1.49%]. In another study carried out by Joshi 1983, brucellosis was found to be 6.08% in humans, 8.7% in cattle and 3.64%

in sheep and goat. During the period of 2003-2013, the central veterinary laboratory [55] tested 5057 serum samples of cattle, buffalo, sheep and goat that were suspected for brucellosis by RBPT, and reported a sero-positivity of 1.48%; in contrast to these findings, the 1425 serum samples tested by indirect ELISA resulted in 0.35% seropositivity. Adhikari [56] reported 2% sero-positivity in goats from Dang, with females having a higher percentage than males. In a recent study by Pandey *et al* [46], 32% of cattle, 13.4% of buffaloes, and 2.6% goats were found to be sero-positive. Brucellosis is endemic in nature and is ever increasing due to changes in the farming practice, increased movement and increased trade of animals [57]. As in correlation with Mantur and Amarnath [58], the widespread use of bulls for the natural insemination might be the factor for endemicity of brucellosis in Nepal. Unlike India and other countries of the world, a religious taboo on the slaughtering of cattle is also contributing to the widespread distribution of brucellosis in animals in Nepal. In addition to this, the free grazing system and frequent mixing of animals of different species may also play a role in fostering a favorable environment for the spread of *Brucella.*

Table 1: Occurence of Brucellosis in animals of Nepal

District	Animal	No tested/ flocks tested	% animals positive	Reference
Solukhumbu	Yak and Nak	31		
Jumla	Yak, Nak, Chauri, Cattle	95	16 (16.84%)	Pyakurel and Mishra [48]
Kathmandu	Cattle,	146	26 (17.47%)	
	Pig,	53	12 (22.64%)	
	Goat	65		
Kaski	Buffaloes			
	Sheep and Goat	24	4 (16.67%)	
Chitwan	Cattle			
Biratnagar	Cattle	72	1 (1.38%)	
Chitwan	Cattle and Buffalo	1069	93 (8.7%)	Joshi [49]
	Sheep and Goat	247	9 (3.64%)	
Kathmandu	Cattle	120	15 (12.5%)	Joshi [59]
Chitwan	Dairy cows	91	3 (3.37%)	Pradhan [51]
Langtang valley	Yaks, Chauri and hilly cattle	74	13 (17.6%)	Lefkowitz *et al,* [54]
Chitwan	Goat	56	3 (5.36%)	Dhakal *et al,* [61]
	Buffalo	35	1 (2.86%)	
			12.03%	Singh [62]

Kathmandu	Pigs	190	41	(21.58%)	Rana [52]
Makawanpur	Goats	41	12	(29.27%)	
Chitwan	Goat	39	2	(5.12%)	
	Sheep	12	2	(14.28%)	
Kathmandu	Goat	37	2	(5.4%)	Poudel [56]
Kailali	Cattle	50	16	(32.00 %)	Pandey et al,[46]
	Buffalo	67	9	(13.43%)	
	Goat	116	3	(2.59%)	

Brucellosis in Humans

Brucellosis is a significant public health problem in Nepal. The first human case was reported in 1979 when the disease was diagnosed in a shepherd from Pokhara by the isolation of *B. melitensis,* who most likely acquired the disease from his sheep. As recorded by various authors [64-66], the incidence of brucellosis in Nepalese in males is between 5.6%-9.42%, while in females it is between 2.9%-6.60%,. Similarly, Joshi *et al.* [59] repeated the research on human brucellosis, which was 4.48% in humans. Joshi *et al.* [57] reported the occurrence of 1.4% in human, which is lower than the finding by Singh 1985, who reported 11.7% occurrence in workers of an abattoir in Kathmandu Valley [Table 2]. This higher incidence of brucellosis among slaughterhouse workers might be due to occupational exposure and poor hygiene of the slaughterhouses. Joshi et al 2007 reported the sero-prevalence of human brucellosis in Kathmandu to be 11.93%, whereas Jackson *et al,[45]* also reported a widespread sero-positivity of brucella in both humans and yak in Dolpa. In total, eighty-four cases of human brucellosis were confirmed from 1991 to 1997 [65] and one hundred and twelve confirmed positive cases of human brucellosis between 1997 and 2002 [66]. Three major species of brucellosis (*B. abortus, B. melitensis,* and *B. suis*) have been reported to be present, although the precise distribution and prevalence of human brucellosis is still not available [66]. Although brucellosis is reportedly endemic in Nepal, neither the distribution nor the economic and public healthimpacts of the disease is well characterized [47]. In Nepal, approximately 90% of people live in villages and come into direct contact with domestic animals on a daily basis [48]; as a consequence, the local people of the villages are at a high risk of acquiring these types of zoonotic diseases [62,66]. Brucellosis has been found to vary by occupation [6.08-11.7%]. The higher sero-positivity of males as compared to the females might be due to the close contact of men with animals, the dynamic nature, and higher mobility than females [66]. The overall prevalence of brucellosis was 110 cases/10^5 inhabitants, which decreased to less than 20 cases/10^5 inhabitants from 2003 onwards [67]. This reduction in reported cases of brucellosis from 2003 onwards might be either due to a lack of disease surveillance, resulting from the under-reporting of the disease in Nepal or an increased awareness among veterinary practitioners and farmers. This higher prevalence of human and animal brucellosis is alarming and significant for animals and public health in Nepal.

Table 2. Occurence of Brucellosis in humans of Nepal

Location	Patient types	Test employed	% Prevalence		References
Kathmandu	Indoor and outdoor patients	PAT,SAT,2ME	57/2117	2.7%	Joshi et al [64]
	Patients admitted to Bir Hospital	Card test	28/200	14%	Rana [44]
Kathmandu		Card test	87/1430	6.08%	Joshi [49]
Kathmandu	Humans of Kathmandu valley	Plate test	3/121	2.47%	Pyakurel and Mishra [48]
		PAT	3/5000	0.06%	Singh et al [62]
	General population	PAT	120/1006	11.9%	Joshi et al [66]
		PAT	25/558	4.48%	Joshi *[57]*
	Patients showing the	PAT	123/1506	8.17%	Joshi et al [66]

	one or more symptoms of brucellosis				
Pokhara	Sheep shepherd	PAT, ELISA	1 case	------	Dickenson and Thaller [68]

Clinical Symptoms

The clinical presentation is non-specific and requires laboratory testing for confirmation. Common presentations (fever, nausea, anorexia, headache, sweating, prostration etc coupled with gastro-intestinal, musculoskeletal, hepato-biliary and meningeo-vascular complications are common [32]. Fever is the most common manifestation followed by the arthritis, sweating and constitutional symptoms [35, 69-71]. It may appear in acute, with an incubation period of 2-3 weeks or sub-acute or chronic cases with an incubation period of weeks to months.

Diagnosis

Brucellosis is an intra-cellular pathogen in cells of the immune system and therefore difficult to diagnose and treat. Definitive diagnosis requires the isolation of the organism from the patient, but is restricted by the fact that *Brucella* spp. are slow-growing intracellular pathogens, whose successful culture from blood decreases as the disease progresses [72,73]. Previous use of antibiotics also adds to the difficulty of culturing the bacillus [74]. As such, successful diagnosis necessitates a careful selection of the best suitable culture method and validation of its performance. The sensitivity of culturing *Brucella* spp. from blood varies from 15% to 70% when compared with clinical evidence of infection and positive serologic results depending on the study and culture method used [73]. Culture provides direct evidence of the presence of the pathogen and is the gold standard, but in the absence of adequate culture facilities, brucellosis might be diagnosed by serologic testing. A variety of serological tests including the Rose Bengal screening test, PAT, SAT, 2-ME, Coombs and ELISA etc are available for the sero-doagnosis of human brucellosis. Most of the serological tests are highly sensitive but they arenot highly specific. Different types of gram negative bacteria such as *Escherichia coli, Vibrio cholera, Escherichia hermanni, Salmonella, Francisella tularensis,* etc., elicit antibody response that cross react with Brucella antigen thus low specificity of serological tests. Though the modern diagnostic techniques with high sensitivity and specificity such as Polymerase Chain Reaction (PCR) have

been introduced, they arenot widely used in field condition and in developing countries.

Diagnosis of brucellosis in field condition relies heavily on serological tests. However, specificity of such tests has been a major cause of concern. Although there are several serological tests such as the agglutination, antigen detection and antibody detection, none of the tests are 100% reliable, so these test results, should be supplemented with the case history, clinical manifestation and other laboratory findings [75-77]. Serological tests should always be interpreted along with the patient history, clinical findings and laboratory findings. At least two serological tests should be used to confirm active infection in animals.

The Rose Bengal Plate test (RBPT) is the most commonly used agglutination test in diagnosis of brucellosis in field condition where there is no possibility to perform SAT. The result of RBPT should always be confirmed by other tests due to its low sensitivity in chronic cases and low specificity in endemic areas [78].

The SAT is the gold standard assay [79-82]. Generally SAT is used first followed by the 2-mercaptoethanol (2ME) test to confirm the result of SAT by excluding the possibility of cross reacting Ig M. Sometimes the result of 2 ME can be mis-interpreted in patients with low levels of IgG [83].

Specific IgM ELISA antibodies are useful in diagnosis of acute brucellosis [75,84] whereas the Coombs test and IgG ELISA are useful in the detection of chronic infection and monitoring of relapsing strains due to their abilities to detect incomplete antibodies[84]. However, dipstick test can be used in patients as a rapid and simple alternative to ELISA IgM test for the serodiagnosis of patients with acute brucellosis [85, 86]. For accurate diagnosis of a suspected case, combination of ELISA IgM and IgG tests with follow-up and monitoring by ELISA IgM and 2-MET might be helpful due to their promising efficiency[87]. In addition, Fluorescence polarization assay [FPA] and immunocapture agglutination for anti-*Brucella* [BCAP] might be alternative choice due to their ability to detect the disease in patients with longstanding evolution of brucellosis. *Brucella* IgM and IgG lateral flow [75] and latex agglutination [88,89] assays have also been found to be rapid and simple along

with high sensitivity and specificity in culture of confirmed cases that are useful for the field condition in remote areas where other methods may not be feasible.

Although the gold standard is isolation of *Brucella* organism from the blood and bone marrow culture, PCR based testing method is a more accurate method for testing the presence of *Brucella,* promising to be more useful and practical [90]. PCR is particularly useful in patients with specific complications such as neurobacillosis or other localized conditions where serological tests fail to address them [91,92] however the sensitivity, specificity and quality control is a matter of concern [3,93,94]. By correcting the drawbacks of conventional PCR, real time PCR has been developed that is less prone to contamination, faster and clinically more useful [95,96]. However, expensive nature of the real time PCR has restricted its use in the field condition and in developing countries.

In short, presumptive diagnosis of brucellosis should be based on patient history and clinical findings. These clinical findings should be combined with the Rose Bengal plate test (RBPT) or SAT, and positive reactors to be confirmed by additional confirmatory tests. Positive ones based on the detection of agglutinating antibodies (RBPT, SAT) should be combined with the detection of non-agglutinating antibodies through tests such as ELISA or Coomb's test.

Treatment of Brucellosis

Treatment of brucellosis in animals isnot effective and infected animal is to be isolated such that further transmission doesnot occur. If feasible, the infected animals should be destroyed and properly disposed. Multi drugs regimens are to be followed for therapy because of high relapse rate reported with monotherapeutic approaches [71, 97, 98]. Treatment failure and relapse rates can be high and depend on the drug combination and compliance [71]. Despite the several studies on antibiotics for the management of brucellosis, no conclusive evidence for optimum antibiotics therapy has been reported so far [71, 98]. The treatment for acute brucellosis recommended by the world health organization (WHO) is doxycycline @100 mg two times a day (BID) orally and rifampin 600 to 900 mg/day per oral (PO) for 6 weeks [99], but this regimen is not to be followed in complicated cases such as the spondylitis and tuberculosis patients [100,101] owing to the possibility of resistant *Mycobacterium tuberculosis*. Rifampin mono-therapy is the major choice for treating brucellosis during pregnancy and combination of sulphamethoxazole- trimethoprim for brucellosis in children [102]. Clinical trials with other antibiotics such as quinolones, macrolides, sulpha-trimethoprim, cotrimoxazole and rifampin have resulted the poor results. Some of the localized form of brucellosis such as endocarditis, meningitis, spondylitis shouldbe dealt with surgery supplemented by the triple antibiotics therapy (Doxycycline, Rifampicin, and Trimethoprim-sulfamethoxazole) [103].What we have to consider here is that the antibiotic regimen has to be selected based on the underlying conditions and the location of the disease. Regarding the case of Nepal, though it has been reported to be endemic, animals are abandoned rather than treatment.

Control

Brucellosis may be controlled by the strict enforcement of a set of measures including test and slaughter, vaccination, sanitation, and movement control [32]. However, the control of brucellosis has proven to be cumbersome and in Hinduism countries the control of bovine brucellosis is limited as the slaughtering of cattle is prohibited. Knowledge of the distribution and spread of brucellosis and their presence in the different livestock and wildlife species is essential for the effective implementation of control measures. A few serological studies have investigated the presence and distribution of brucellosis in livestock and human in Nepal and the risk of transmission to the human population.

Lack of awareness among the farmers, and limited vaccine availability has made the situation favorable for the endemicity of brucellosis in Nepal.Thus; animal owners should be made aware about the economic impact, health impact and importance of vaccination of their livestock. Public health education should emphasize on food hygiene and occupational hygiene. Avoiding or discouraging using raw milk and dairy products, a strict procedure of protective and safety measures of health workers will help prevent brucellosis in human population. Details of preventive and control measures have been discussed in recommendation section below:

Recommendations

Some of the recommendations for prevention of brucellosis in Nepal are as follows:

Increase in public awareness by awareness campaign:

Efforts should be made by the government and the concerned sector for raising awareness of the disease and its impact on public health. The first weapon of disease prevention is minimization of exposure, which can be achieved by the educating the high risk groups including farmers and others from similar occupational category. Clothes and other protective measures should be followed during handling of   animals. Training should be provided for the farmers and livestock attendants for effective sanitary and hygienic management practices during handling of animals and animal products such as aborted fetus and other body secretions. Thus, in underdeveloped countries like Nepal, where eradication in animals (through the mass vaccination and/or elimination of infected animals) is not feasible, prevention of human infection should be based on public awareness, food safety measures, laboratory safety and occupational hygiene.

Food hygiene

Since *Brucella* is readily killed by heat treatment, boiling of milk and heating of milk products at 80-85 degree centigrade will kill these bacteria and makes milk safe for human consumption. The feeding habits of raw and uncooked meat and milk need to be understood in its complete sense similar to the methods of preparation and addition of spice and herbs. Likewise, cultural practices such as direct milk drinking from the udder, uncooked cow's milk and urine mixed together to make elixir should be carefully studied for any chances of creating infection. In Nepal, we have practice like drinking raw blood from standing yak and, health concerns of such practices have to be established before we advocate its modification.

Control of brucellosis in animals by regular surveillance, screening and immunization

Regular surveillance is necessary as it is one of the important steps for preventive and control measures. Cost effective surveillance method includes regular testing of bulk quantities of milk [104] and RBPT as well as the PAT test to screen for positive animals. Although the MRT is not an effective test for testing the milk of sheep and goats due to fatty nature, other alternatives such as the RBPT and PAT can be used effectively[105]. Strict implementation of quarantine and sero-surveillance is an utmost necessity [106,107]. Detailed epidemiological investigation of the three pillars of the disease should be conducted across the country to identify the associated risk factors for the occurrence and endemicity of the disease. Immunization of animals with vaccines is the utmost need for prevention and control. Rev1, a modified live virus vaccine can be used in small ruminants at three to four months of age providing immunity for three to five years [108,109]. Strain 19 is commonly used for the prevention of brucellosis in cattle. These vaccines are used to vaccinate calves between four to twelve months. The vaccination of animals is an important choice for the prevention, control and eradication of brucellosis [104,110-114].

Maintaining healthy contact with animals

Since the sharing of water points for drinking and grazing lands are important risk factors for transmission of brucellosis, avoiding the mixing of cattle and other animals as well as raising a replacement heifer within a herd might be an important step for control and prevention of brucellosis in humans and animals. Screening of animals before purchasing and entering them into a herd, proper disposal of aborted materials and isolation of animals in parturition help to reduce the transmission of brucellosis across the herd [111].

Testing and slaughtering of infected animals

If the regular vaccination of animals against brucellosis is not practiced then the test and slaughter method can be used as the cost effective measures [7,108]. For the under-developed countries like Nepal, the test and slaughter method is difficult due to the cost involved in the slaughter of animals [112,115,116] and the religious taboo existing in the country. The control of disease in animals in Nepal should depend first on the mass vaccination of all animals for few years till the incidence of infection decline up to 1%, and only then one can count on the test and slaughter of infected animals, as it will be non-expensive. Therefore, full cooperation of government and other stakeholders is necessary for this method to be successful.

Conclusion

Like other underdeveloped and developing countries of world, the situation of brucellosis is alarmingly serious herein Nepal. There is a urgent need of extensive research on epidemiolical picture and public health impacts. Control of brucellosis in animals and reducing transmission to humans should be instigated. Vaccination and awareness program should be

strengthened by veterinary and para-veterinary services.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

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