Leptospirosis in Himachal Pradesh

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Abstract
Background: Leptospirosis is an uncommon disease in North India; but with recent increased prevalence, especially in Himachal Pradesh (HP). No prospective information is available from the state of Himachal Pradesh.

Aim & Objectives: To study the phenomenon of Leptospirosis in the North Indian state of Himachal Pradesh and thereby (i) determine its prevalence amongst patients with Fever of Unknown Origin (FUO), (ii) evaluate and understand the clinico-epidemiologic pattern of leptospirosis in HP, and (iii) evaluate the utility of Modified Faine’s Criteria for diagnosing leptospirosis.

Methodology: Prospective study conducted in patients with FUO over a 1-year period (June 2006- May 2007). The patients with a strong clinical suspicion of a diagnosis of leptospirosis were subsequently identified by the treating physicians. Their blood samples subjected to ELISA (as per manufacturer’s instructions) and MAT serology. For the ELISA serology positive cases, Modified Faine’s Criteria were administered to arrive at a presumptive/definitive diagnosis of leptospirosis.

Results: Amongst 100 cases of FUO, 26% had a high clinical suspicion of leptospirosis. These 26 cases were subjected to IgM ELISA for leptospirosis; 7/26 (27%) testing positive. The 7 MAT samples got contaminated; hence results unavailable. 5/7 patients developed FUO in the rainy season and 2/7 in the non-rainy season. The mean age was 35 years; male to female ratio=1.3:1; all were from a rural background.

The epidemiological and clinical profile had some significant differences to that reported in the earlier reported studies from the same region.

Using Modified Faine’s Criteria, all 7 cases received a presumptive diagnosis of leptospirosis i.e. total score ≥26 (i.e. 100% Positive Predictive Value). However, without availability of serological measures; 2/7 cases were not receiving the presumptive diagnosis of leptospirosis i.e. false-negative’ value of 28%; these getting rightly diagnosed/classified using Original Faine’s Criteria.
Conclusions: Leptospirosis is not as uncommon in HP as previously thought. There appears to be a possible differing clinico-epidemiological profile that needs further evaluation. There appears to be a role of application of both Original and Modified Faine’s Criteria in non-rainy and rainy regions of HP (and possibly even in other parts of North India). There is a need to carry out epidemiologically designed, large-sample prevalence studies on FUO and leptospirosis utilizing both sets of Faine’s Criteria.

Summary: This study was conducted in the North Indian state of Himachal Pradesh with the aim of finding prevalence of leptospirosis in patients of fever of unknown origin (FUO). FUO is a complex entity with over 200 causes; leptospirosis being one of the important ones. However, leptospirosis is a neglected diagnosis in this part of India due to lack of awareness among physicians and lack of diagnostic modalities. Serology (using IgM ELISA) is the most common method to diagnose leptospirosis, as the microorganism is difficult to cultivate. Clinically, Modified Faine’s criteria (especially developed for the Indian setting) are useful in diagnosis of leptospirosis. In this study, of 100 cases with FUO, 26 cases with strong clinical suspicion of leptospirosis were subjected to ELISA; 7/26 (27%) were positive. Modified Faine’s criteria were applied to these confirmed 7 cases; only 5/7 met the modified criteria (and 2/7 met the original Faine’s criteria). To conclude, leptospirosis is prevalent in North India, and both the Original and Modified Faine’s criteria may have a role in arriving at a clinical diagnosis for patients presenting from non-rainy and rainy regions respectively.

Keywords: Leptospirosis, Faine’s criteria, India.

Introduction
Leptospirosis has been known to exist in India since 1931\(^1\) with widespread prevalence in all parts except Northern India\(^1, 2\). It is only in the last decade that reports have emerged indicating presence and possible increasing prevalence of leptospirosis in various parts of North India; detailed elsewhere\(^1,2,3\).

To the best of our knowledge, there are only few studies that have reported leptospirosis from Himachal Pradesh\(^1,3\). These were, however, retrospective studies and hence associated with their own understandable limitations.

Additionally, there are no prospective studies that have been conducted on examining the presence of leptospirosis in Himachal Pradesh (HP).

We studied leptospirosis as part of a larger study, which was conducted with the aim of identifying various infectious causes of Fever of Unknown Origin (FUO).

Aim
To study the phenomenon of Leptospirosis in the North Indian state of Himachal Pradesh.

Objectives
1. To determine the prevalence of leptospirosis amongst patients with FUO
2. To evaluate and understand the clinico-epidemiologic pattern of leptospirosis in HP
3. To evaluate the utility of Modified Faine’s Criteria for diagnosing leptospirosis.

Materials and Methods
Setting: Departments of Microbiology and Internal Medicine at Indira Gandhi Medical College (IGMC) and Hospital, Shimla, H.P.

IGMC is the largest multi-speciality hospital developed since 1966 catering to the needs of residents of HP. It is a 1000-bedded hospital with nearly 140 beds dedicated to Internal Medicine. The turnover in IGMC is high with average length of stay being 9 days and a bed occupancy rate of approximately 93% (http://www.igmcshimla.org/AnnualBulletin.htm).

Time Frame: The present study was conducted over a period of one year from June2006 to May 2007.

Design: Prospective.

Sample: The sample was identified in a purposive manner from all patients of any age and gender, admitted under Department of Internal Medicine as per inclusion and exclusion criteria.
**Inclusion Criteria:** Presence of FUO as defined by Petersdorf & Beeson (4). **Exclusion Criteria:** Uncooperative, non-consenting patients.

**Procedure:** All patients meeting the above mentioned selection criteria and based on information provided by the referring physician were identified by the principal author (NJ). Informed consent was obtained. FUO was confirmed as per above-mentioned case definition criteria.

The patients with a strong clinical suspicion of a diagnosis of leptospirosis were subsequently identified by the treating physicians. Their blood samples were subjected to ELISA as per manufacturer’s instructions. Following this, the blood samples were also sent for MAT testing to the Regional Medical Research Centre (ICMR), Port Blair (as detailed below).

For the serology positive cases, we administered Modified Faine’s Criteria, which are used to arrive at a presumptive/definitive diagnosis of leptospirosis\(^5\).

**Laboratory Measures**

1. **Enzyme-linked immunosorbent assay (ELISA)** - ELISA for *Leptospira* immunoglobulin (Ig) M antibody (Serion Immunodiagnostica GmbH, Würzburg, Germany). As per manufacturer’s specifications, the sensitivity, specificity, positive predictive value, and negative predictive value of this kit are 96%, 97%, 90%, and 99%, respectively\(^2\).

   The tests were performed according to instructions given in the manual provided with the kit.

2. **Microscopic Agglutination Test (MAT)** – MAT is a serovar specific test that is ideally performed on paired samples, drawn a few days apart. MAT is deemed to be a dependable diagnostic tool for leptospirosis (next only to isolation) by providing serovar specific diagnosis\(^2\). The criteria for confirmation of leptospirosis include-seroconversion or four-fold rise in titre in paired samples (Vijaychari P, personal communication; 6). However, MAT is associated with its own significant limitations\(^1,2\).

**Results**

100 cases of FUO, as defined by Petersdorf & Beeson (4), were taken up during the study period. Of these 100 cases of FUO, high clinical suspicion of possible diagnosis of leptospirosis (based on their socio-clinical profile) was considered by the treating physicians in 26 i.e. 26% of the sample. These 26 cases were subjected to IgM ELISA for leptospirosis; 7/26 (27%) testing positive. Additionally, MAT was performed in these 7 cases. But, as the samples got contaminated in transit to the reference laboratory, the results were not incorporated.

5 of these 7 patients developed FUO in the rainy season (August-September), and 2 in the non-rainy season (November-December). Sampling for IgM and MAT was carried out within 48 hours of admission.

The mean age of the patients was 35 (Range = 26-53) years. Male to female ratio was 1.3: 1. All were from a rural background.

Two cases showed co-infection with Scrub typhus, as a high titer (>640) was seen by using the Weil Felix test along with positive IgM ELISA for scrub typhus.

Table 1 details the epidemiological risk factors for the 7 cases.

Table 2 details the clinical features for the 7 cases.

Table 3 details the laboratory findings for the 7 cases.

Using all the available socio-clinical-epidemiologic information, these 7 cases were assessed on, and scored, using Modified Faine’s Criteria (5) [Table 4].
Table 1: Epidemiological Risk Factors

| Parameters                               | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 | Case 7 |
|------------------------------------------|--------|--------|--------|--------|--------|--------|--------|
| Wet Living Conditions                    | +      | +      | +      | +      | +      | -      | -      |
| Lack of Protective Footwear              | +      | +      | -      | +      | +      | +      | +      |
| Dwelling infested with rats              | +      | +      | +      | +      | +      | +      | +      |
| Working in Farm Lands                    | +      | +      | +      | +      | +      | +      | +      |
| Contact with animals, especially cattle | +      | +      | +      | +      | +      | +      | +      |
| Bathing in Public Places                 | -      | -      | +      | +      | -      | +      | +      |
| h/o unprotected contact with dirty stagnant water | -      | -      | -      | -      | -      | -      | -      |
| Alcohol addiction                        | -      | -      | -      | +      | -      | +      | +      |
| Smoking                                  | +      | -      | +      | -      | +      | -      | -      |
| Occupation                               | Farming | Farming | Farming | Farming | Farming | Farming | Farming |

Table 2: Clinical Features

| Parameters                               | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 | Case 7 |
|------------------------------------------|--------|--------|--------|--------|--------|--------|--------|
| Headache                                 | +      | -      | +      | -      | +      | +      | +      |
| Fever                                    | +      | +      | +      | +      | +      | +      | +      |
| If fever, temp 39C or more               | +      | +      | +      | +      | +      | +      | +      |
| Conjunctival suffusion (bilateral)       | +      | +      | +      | -      | +      | +      | +      |
| Meningism                                | -      | -      | -      | -      | +      | -      | -      |
| Muscle Pain (esp calf muscle)            | +      | +      | +      | +      | +      | +      | +      |
| Conjunctival suffusion+Meningism+Muscle Pain | -      | -      | -      | -      | -      | -      | -      |
| Jaundice                                 | +      | +      | +      | +      | +      | +      | +      |
| Albuminuria or Nitrogen retention        | +      | +      | +      | +      | +      | +      | +      |

Table 3: Laboratory Parameters

| Parameters                               | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 | Case 7 |
|------------------------------------------|--------|--------|--------|--------|--------|--------|--------|
| Leucocytosis>11000/cu.mm                  | +      | +      | +      | +      | +      | +      | +      |
| Anaemia (Hb<10.0 gm/dl)                   | +      | +      | +      | +      | +      | +      | +      |
| D-dimer positive                         | -      | -      | -      | -      | -      | -      | -      |
| Increased Prothrombin Time               | -      | -      | -      | -      | -      | -      | -      |
| Thrombocytopenia (<100000/cu.ml)          | +      | -      | +      | -      | -      | -      | -      |
| Deranged ALT/AST                          | +      | +      | +      | +      | +      | +      | +      |
| Hyperbilirubinemia                        | +      | +      | +      | +      | +      | +      | +      |

Table 4: Modified Faine’s Criteria

| Parameters                               | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 | Case 7 |
|------------------------------------------|--------|--------|--------|--------|--------|--------|--------|
| Part A: Clinical Data                    |        |        |        |        |        |        |        |
| Headache                                 | 2      | -      | 2      | -      | 2      | 2      | 2      |
| Fever                                    | 2      | 2      | 2      | 2      | 2      | 2      | 2      |
| If fever, temp 39C or more               | 2      | 2      | 2      | 2      | 2      | 2      | 2      |
| Conjunctival suffusion (bilateral)       | 4      | 4      | 4      | -      | 4      | 4      | 4      |
| Meningism                                | -      | -      | -      | 4      | -      | -      | -      |
| Muscle Pain (esp calf muscle)            | 4      | 4      | 4      | 4      | -      | 4      | -      |
| Conjunctival suffusion+Meningism+Muscle Pain | -      | -      | -      | -      | -      | -      | -      |
| Jaundice                                 | 1      | 1      | 1      | 1      | 1      | 1      | 1      |
| Albuminuria or Nitrogen retention        | 2      | 2      | 2      | 2      | 2      | 2      | 2      |

Part B: Epidemiological Factors

| Parameters                               | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 | Case 7 |
|------------------------------------------|--------|--------|--------|--------|--------|--------|--------|
| Rainfall                                  | 5      | 5      | 5      | 5      | 5      | -      | -      |
| Contact with contaminated environment    | -      | -      | -      | -      | -      | -      | -      |
| Animal Contact                           | 1      | 1      | 1      | 1      | 1      | 1      | 1      |

Part C: Bacteriological and Laboratory Findings

| Parameters                               | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 | Case 7 |
|------------------------------------------|--------|--------|--------|--------|--------|--------|--------|
| Isolation of Leptospira on culture*      | -      | -      | -      | -      | -      | -      | -      |
| ELISA IgM positive                       | 15     | 15     | 15     | 15     | 15     | 15     | 15     |
| MAT rising titre (paired sera)*          | -      | -      | -      | -      | -      | -      | -      |
| TOTAL SCORE                              | 38     | 36     | 38     | 36     | 38     | 29     | 33     |

(*) = Not Present; hence- not scored
*Not carried out; hence-not applicable

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Discussion

Objective 1: Prevalence of Leptospirosis

As mentioned earlier, this is the first such clinico-serological study on leptospirosis from Himachal Pradesh in which the data was prospectively collected. We shall be discussing various characteristics of the 7 positive cases in detail, as this should help in developing a better understanding from both clinical and epidemiological perspective.

Leptospirosis has been reported to be endemic in all parts of India, except Northern India (1). In their study, Sethi et al (1) found a high percentage of patients from Himachal Pradesh. It is difficult for us to compare the results in this study with them, primarily due to the design of the studies. It is not possible to compare our results with those obtained by Chauhan et al (3) from the same centre, as they did not report any prevalence figure. However, there appears to be a possibility of leptospirosis being more common in H.P. than earlier thought as Chauhan et al (3) detected 13 cases in a 3-month period.

However, 27% (7/26) positivity of all FUO cases with a high clinical suspicion of leptospirosis is too high a figure to ignore. Our results are in keeping with the concerns raised by Chauhan et al (3) that leptospirosis may be developing new endemic pockets in H.P. In fact, Chauhan et al (3) have recommended the need for appropriate Institute-based laboratory facilities to perform confirmatory tests.

Objective 2: Epidemiological-Clinical-Laboratory Pattern

The identified patients in our study were comparable to those from Sethi et al (1) on parameters of age, gender, locality background. Although Sethi et al (1) did not specifically provide such information on their sub-sample from Himachal Pradesh, but similarity to their overall sample indicates that leptospirosis seen by us is not different (on these parameters) to that reported from other parts of Northern India. However, in another study from the same Institute (3), leptospirosis was found to be higher in females (77%). Additionally, they reported higher age of presentation (44 years) but all patients were from a rural background (as in our study).

It has been highlighted about the potential change in course of the epidemiological and clinical pattern for leptospirosis (8), and this pattern may vary from region to region (1). Hence, we felt it imperative to compare and contrast our findings with the two available studies (that had patients from the state of H.P.) from Chandigarh (1) and Simla (3).

On evaluating the profile related to ‘epidemiological risk factors’, the least common so identified were- h/o unprotected contact with dirty stagnant water followed by alcohol addiction and smoking. These findings are in contrast to those by Sethi et al (1) who reported the least common to be- lack of protective footwear (being barefoot) and high percentage of people having contact with dirty water. However, they did report low presence of alcohol addiction and smokers in their sample. The reasons for the same are difficult to explain, but it is most likely due to patients coming from other states, which may have influenced the pattern so seen in their study. Although Chauhan et al (3) had reported ‘contact with animals, rainfall, contaminated environment’ in all (13; 100%) of their sample, it is difficult for us to compare with their results as the three environmental parameters (i.e. rainfall, contact with animals, and contact with contaminated environment) had been clubbed together by them.

The clinical profile was also somewhat dissimilar to that reported by Sethi et al (1) i.e. high percentage of patients with headache and myalgia, and all suffering with conjunctival suffusion in our study. Findings related to jaundice were similar to that by Sethi et al (1) and different from that reported from South India (9). Meningism was very uncommon, in contrast to that reported in studies from both North India (1) and South India (9). This dissimilarity is more likely to be a product of the small sample size in our study.
Surprisingly, the clinical profile in our cases differed to a significant extent from that reported by Chauhan et al\(^3\) from the same Institute. We had higher percentage of patients suffering with Fever>39\(^\circ\)C (100% vs 54%), jaundice (100% vs 77%), conjunctival suffusion (87% vs 54%) and a lower percentage of patients with headache (72% vs 92%), meningism (13% vs 54%). These dissimilarities are hard to explain but maybe arising out of methodological differences and differing time-frame for patient intake.

Interestingly, the profile so obtained with the laboratory parameters was quite similar to those seen in the retrospective study by Sethi et al\(^1\), though there was no case with increased prothrombin time and D-dimer positivity.

Hence, overall it can be seen that the epidemiological and clinical profile of our patients had some significant differences to that reported from the earlier reported studies from the same region\(^1,3\) and from South India\(^8,9\). Differences wrt studies from South India are probably easier to understand due to markedly differing environmental and seasonal conditions across these two zones. As regards differences compared to previous studies from North India, it may be possible due to (i) larger sample with more heterogeneity due to catchment area being whole of North India, rather than localized to Himachal Pradesh\(^1\), or (ii) an actual difference in pattern of leptospirosis manifestation in Himachal Pradesh\(^3\). It is difficult to speculate further, and this issue can be best addressed by conducting further studies with a larger sample size and controlling for various confounding factors.

**Objective 3: Application of Modified Faine’s Criteria**

Modified Faine’s Criteria are used for diagnostic purposes\(^1,3\). However, as per design of our study, we had adopted the 2-phase approach i.e. initial clinical suspicion followed by serological testing for leptospirosis. In order, to evaluate whether cases identified by this approach could be appropriately classified /diagnosed by using a criteria-based approach, we subjected the available information to the Modified Faine’s Criteria\(^5\).

Before proceeding further, it may be pertinent to briefly discuss about the Modified Faine’s criteria. The original criteria\(^5\) incorporated clinical, epidemiological and laboratory data.

Modifications were carried out in the areas of epidemiological and laboratory sections; these being necessitated especially for the Indian setting due to (i) high degree of rainfall and association of leptospirosis thus seen, and (ii) complications associated with the MAT test\(^5\). A score of 20-25 indicates ‘possible leptospirosis’ and a score of >26 indicates ‘presumptive leptospirosis’.

It was seen that all 7 cases received a presumptive diagnosis of leptospirosis i.e. total score >26. Hence there was 100% Positive Predictive Value for the Modified Faine’s Criteria i.e. all clinical-serologically identified positive cases with leptospirosis were correctly identified. The inherent advantage associated with this result is that physicians can use these criteria to arrive at a possible or presumptive diagnosis of leptospirosis. This argument carries further credence, as the common clinical features (fever, headache, conjunctival suffusion, myalgia, jaundice) that are seen in (or raise a clinical suspicion of) leptospirosis are part of the Modified Faine’s Criteria. Leptospirosis is an illness in which the serological results are available not before day 5 of the onset of the illness, and prior to that, clinicians have to rely on the available clinical and epidemiological data\(^3,5\). Using Parts A and B of the Modified Faine’s Criteria can be a useful checklist and evidence-based approach to back-up a clinical diagnosis of suspected leptospirosis. By doing so, in the initial 5 day period, an empirical trial of antibiotic treatment for ‘suspected/possible’ leptospirosis can be thereby safely initiated. Additionally, this approach can also have potential implications in being able to advise and conduct appropriate serological tests for FUO, thereby leading to less wastage of kits or ordering of generalized serological tests for FUO patients.
As earlier mentioned, there is considerable literature highlighting the association of rainfall with leptospirosis in India, and the development-cum-application of Modified Faine’s Criteria (1-3, 5). However, it was interesting to note that there were 2/7 (28%) cases of FUP who developed leptospirosis in the non-rainy season. Application of the Modified Faine’s Criteria for these two cases (nos 6,7) gave scores of 29 and 33 respectively. Table 1 show that understandably they scored ‘zero’ for the parameter of ‘rainfall’ (Part B of criteria). Going by our earlier assertion, if the clinicians had to use Parts A+B of the Modified Faine’s Criteria for arriving at a ‘possible’ diagnosis (score of 20-25) in the first week of illness, then the score would have been 14 and 18 respectively (due to removal of 15 points for the ELISA positivity). Hence, these cases would have been missed out i.e. false-negative’ results for leptospirosis using Modified Faine’s Criteria. This limitation has been previously highlighted that - it is difficult to make a diagnosis of leptospirosis in non-monsoon months using the modified criteria\(^{(5)}\). There are implications for this limitation as in that- all parts of Himachal Pradesh do not receive heavy rainfall in monsoon/rainy months e.g. Trans-Himalayan/Spiti zone receives very limited rainfall leading onto dry and cold weather (http://www.himachalpradesh.us/geography/himachal_climate.php). A closer look at the modifications to the original scale highlight the fact that the parameter of ‘contact with animals or contact with known contaminated water’ was modified into 3 parameters (rainfall, contact with contaminated environment, animal contact).

‘Animal Contact’ was present in both these cases. As the clinical parameters are unchanged in both scales, if we apply the original scale\(^{(7)}\), then we get scores of 23 and 27 (for cases 6 and 7 respectively) thereby correctly classifying them into a diagnosis of ‘probable’ and ‘presumptive’ leptospirosis. Hence, it may be necessary to consider use of either of these two scales/criteria, depending upon the presence (or absence) of rainfall in the season when a patient with FUO presents to the hospital. It may, therefore, be important to evaluate the sensitivity, specificity, and PPV\(^{(5)}\) of both scales in a historically non-endemic area (e.g. H.P.) where rainfall is not a key environmental factor.

**Limitations**
The results provided and discussion thereof, needs to be interpreted with caution, as the sample size is very small and we were unable to carry out a rigorous testing of the Modified Faine’s Criteria. Additionally, as this was a time-bound study, we were restricted in terms of a longer duration of follow-up to assess the outcome of the positive cases. Lastly, we were unable to analyze the ‘serology negative’ cases for testing out the applicability of Faine’s Modified Criteria.

**Conclusions**
This study is the first of its kind from Himachal Pradesh that was prospectively conducted amongst patients with FUO and explores various clinical, epidemiological, serological aspects of leptospirosis. Additionally, it explores the applicability and utility of a rating-cum-diagnostic scale (Modified Faine’s Criteria) thereby attempting to provide direction towards evidence-based practice for managing cases with FUO and suspected leptospirosis. Hence, despite the above-mentioned caveats, there should be an attempt to carry out epidemiologically designed, large-sample prevalence studies on FUO and leptospirosis in order to be able to address the issues highlighted. This study makes a case for the retention and application of the Original Faine’s Criteria in the non-rainy Indian regions. It also adds to the existing meager Indian literature on leptospirosis, and hopefully shall generate interest for future research.

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