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# Annual incidence of visceral leishmaniasis in an endemic area of Bihar, India

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**Summary** The study presents the findings of a population-based survey of the annual incidence of visceral leishmaniasis (VL) in the rural areas of one VL-endemic district in Bihar, India. Stratified multi-stage sampling was applied in the selection of blocks, villages, hamlets, and households. We screened 15 178 households (91 000 individuals) in 80 villages in 7 of 27 administrative blocks of the district, East Champaran. We identified 227 VL cases that occurred in the past 12 months: 149 treated individuals who survived, 14 who died from VL, and 64 active cases. The high-incidence stratum had an estimated incidence of 35.6 cases per 10 000 persons per year (90% CI: 27.7–45.7). The annual incidence rate in the medium stratum areas was 16.8 cases per 10 000 (90% CI: 9.3–30.6). The combined annual incidence rate for the high and medium areas combined was 21.9 cases per 10 000 per year, (90% CI: 14.0–34.2). The Government of India's VL elimination goal is to reduce the VL incidence to one case per 10 000 at the sub-district level; thus, a 35-fold reduction will be required in those areas with the highest VL incidence.

**keywords** population-based survey, visceral leishmaniasis, annual incidence rate, VL-endemic district, Bihar, stratified multi-stage sampling

## Introduction

The visceral leishmaniasis (VL) endemic area of South Asia extends from its epicentre in Bihar in north-eastern India to the adjacent Indian states of Jharkhand, West Bengal, and Uttar Pradesh, into south-eastern Nepal, and central and western Bangladesh. *L. donovani*, the parasite responsible for VL in South Asia, is transmitted by *Phlebotomus argentipes*, an endophilic sandfly vector that resides in human and animal dwellings in densely populated agricultural villages. In 2005, the Governments of India, Bangladesh, and Nepal agreed to participate in a regional VL elimination program to reduce the incidence of VL to one case per 10 000 population by 2015.

Currently, estimates of the number of VL cases in India are based on official government reports. The passive reporting captures individuals who seek VL treatment in government health facilities: primary health centres, block health centres, and district hospitals. Patients treated in the private sector, reported to comprise as much as 70–80% of the total (UNICEF *et al.* 2007), and those who do not seek treatment are not captured in these reports. It is widely acknowledged that official figures in India and elsewhere considerably underestimate the true burden of VL (Desjeux 2004; Bern *et al.* 2008). Two population-based studies of VL incidence in highly endemic sub-districts of Bihar, India, estimate the annual VL incidence as 24.9 (Singh *et al.* 2006) and 57.1 (Singh *et al.* 2010) per 10 000 population; they suggest that official figures underestimate VL incidence by a factor of eight (Singh *et al.* 2006) and four (Singh *et al.* 2010). The current study was conducted to provide population-based estimates of the number of VL cases in the district of East Champaran, an endemic area of Bihar that has experienced an increase in VL cases. The study is intended to guide estimates of the resources required to support VL elimination campaigns and to establish a baseline to from which to assess the impact of program interventions.





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## Methods

### Study district

East Champaran (Purba or Purbi Champaran) district is in northwest Bihar, bordering Nepal. It was selected as the study district because government VL reports indicated an increasing number of cases from 2002 to 2005 and there were no ongoing VL clinical trials, epidemiological studies, or intervention projects in the district at the time of selection. According to the 2001 census of the Government of India, the population of East Champaran was 3 939 773, with 93% being rural (Census of India 2001). The district has 27 administrative blocks. As VL is predominantly a disease of rural areas, the study excluded the seven urban towns.

### Sampling method

A stratified multiple stage sampling procedure was adopted to select survey households. Data were collected in two phases, the first in December 2006 and the second in April 2007.

The blocks of East Champaran were grouped into three strata: 'high,' 'medium,' and 'low,' consisting of three, nine, and fifteen blocks, respectively. Block classification was based on the number of VL cases in the 2005 and 2006 monthly district reports and supplementary information collected from primary health centres by the survey team. For Round 1, two blocks were chosen at random from each stratum. For Round 2, the third block in the high-incidence stratum was added to provide additional cases for a companion study of the economic impact of VL (Figure 1).

The study villages were selected by simple random sampling. In the first round, 10 villages were chosen from each of the high-incidence blocks, six villages from each medium incidence block, and four villages from each low-incidence block. In the second round, another 10 villages were selected from each of the two original high-incidence blocks and 20 villages were selected from the added third high-incidence block. Altogether, 80 villages were selected, including 20 from each high-incidence block (Table 1).

The sampling plan included selection of 200 households in each selected village (all households if fewer than 200).



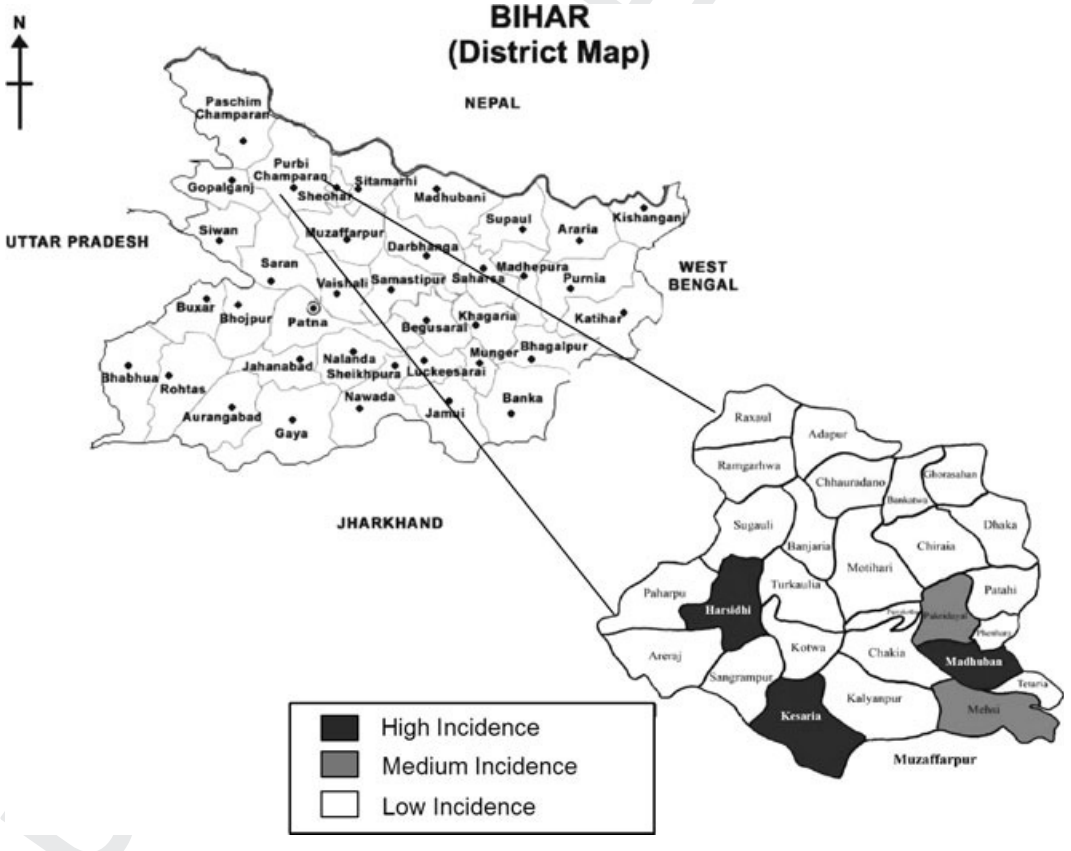


**Figure 1** Bihar map showing all districts and study district of East Champaran (Purbi Champaran) with blocks.



 

**Table 1** Number of blocks, villages, and population in rural Bihar and household response rate by incidence stratum

| Incidence stratum | No. of blocks | No. of blocks selected | No. of villages in selected blocks | No. of villages selected | Population study villages 2001 census | No. of households selected for survey | No. of households surveyed | Household response rate |
|---|---|---|---|---|---|---|---|---|
| High | 3 | 3 | 163 | 60 | 153 257 | 11 344 | 10 374 | 95 |
| Medium | 9 | 2 | 492 | 12 | 33 934 | 2286 | 2064 | 92 |
| Low | 15 | 2 | 624 | 8 | 19 576 | 1548 | 1424 | 92 |
| Total | 27 | 7 | 1279 | 80 | 206 767 | 15 178 | 14 233 | 94 |

A field supervisor consulted village leaders to identify the hamlets in villages with more than 200 households and to estimate the number of households in each hamlet. All selected villages and hamlets were mapped. A total of 420 hamlets were identified in 80 villages. An equal proportion of households was sampled in each hamlet of a village and section of the hamlet. A fixed number of starting points were designated for each hamlet based on the population size; multiple starting points help ensure broader geographical coverage of the sampling unit. Interviewers proceeded in a clockwise direction from the designated started point until the requisite number of households was selected.

### Survey implementation

A trained interviewer visited each selected household and asked the household head or a responsible adult whether any of the household members was currently suffering from VL, experiencing a fever for more than 2 weeks, had been diagnosed with VL, died from VL, or died from an illness with a fever lasting longer than 2 weeks in the last 12 months. All individuals who met at least one of these criteria were considered possible VL cases. The possible cases or informants (if the case had died or was unavailable) were invited for a clinical interview conducted the same day by the survey team's medical doctor. For children, the parent or guardian was invited to bring the child to the doctor. Prior to the clinical interview, an informed consent form was signed. The interview comprised questions about the person's medical history: symptoms, diagnostic tests conducted, diagnosis received, treatments, and a review of available medical records. Possible cases were given a rapid diagnostic test for VL (rK39 test; InBios, USA). The rK39 test is based on the detection of the subject's antibodies to an antigen of *Leishmani*a. The test has a reported sensitivity of 98–100% for active VL in this population, although up to 15% of healthy inhabitants of endemic areas may have a positive test (Chappuis *et al.* 2006; Sundar *et al.* 2006). If the individual was suffering from fever at the time of the interview, the spleen was palpated. The survey doctor either diagnosed a current case of VL or a case in the past 12 months based on a combination of: the clinical interview, the diagnosis received at the time of the illness, diagnostic test results, medications prescribed, reported signs and symptoms, and the rk39 test result.

### Weighting and analysis

A weight was assigned to each household member so the weighted incidence estimates would reflect those in the population. The weights were calculated in two steps: computation of sampling weights to reflect differential probabilities of selection and adjustment of the sampling weights to match characteristics of the sample to those known from the 2001 census of India (Groves *et al.* 2004).

### Sampling weight

The sampling weight was calculated as the inverse of the probability of selecting each sampled household. This probability was computed by multiplying the probabilities of selection at each stage of the selection: block, village, and household. As information was obtained about all members of a household, each individual received the sampling weight assigned to the household. The only uncertainties in these calculations were those concerning the household selection probabilities. Unfortunately, records were not kept of the systematic sampling intervals or of the exact household counts in each hamlet. These probabilities were estimated by dividing the number of selected households by an estimate of the total number of households in the village for all but the seven villages where all households were selected. This estimate was made by projecting a growth rate of 1.8% per year from the 2001 census total.

### Post-stratification rates

To better represent the district, the sampling weights were normalized so that weighted totals would reproduce two

population distributions from the 2001 census for the medium-incidence stratum and for the three high-incidence blocks combined. These distributions were the age-gender proportions and the proportions of people residing in villages of 2000 or more residents. The adjustment process is known as 'raking' and was implemented by the Stata command *survwgt*, written by Nicholas Winter, University of Virginia (Deming & Stephan 1942; STATA 2007). The data were initially edited in SAS©. Construction of analysis data sets and all analyses were performed with STATA, Release 10.

## Results

The study sample consisted of 15 178 households in 80 villages in 7 administrative blocks of East Champaran. Table 1 shows the number of blocks and villages and the 2001 census populations of those villages for the three incidence strata. According to the 2001 census, 75% of the sample villages had more than 1000 inhabitants (median 1887 inhabitants; range 46–14 808), with a median of 282 households (range: 4–2255). The median household size was 6.3 people (range: 4.2–11.5). The study villages comprised about 5% of the villages of rural East Champaran and about 6% of East Champaran's rural population. Over one-third of individuals in East Champaran's high-incidence stratum were in selected households.

### Study households

The 15 178 selected households constituted 64% of all households in the study villages. Interviews were obtained in 14 223 households (94%) with 91 009 individuals; the remainder was unavailable at the time of interview.

A total of 471 individuals identified as possible cases were referred for the clinical interview. Of these, 450 individuals (96%) from 398 households (95%) reported. Nine individuals who were not referred appeared and were screened. None of these nine was a VL case. The most common reasons for referral in the high-incidence stratum were possible current VL or VL in the past 12 months. In the medium- and low-incidence strata, the reasons were fever for 15 days currently or in the past 12 months.

### Visceral leishmaniasis among referred individuals

VL was diagnosed in 227 (50%) of the 450 people evaluated at the clinical interview: 64 current cases and 163 past cases, of whom 14 had died. The case disposition of referred individuals is summarized by stratum in Table 2. In the high-incidence stratum, 63% of referred cases were diagnosed as VL cases. The corresponding percentages in the medium- and low-incidence strata were 25% and 4%, respectively. Over 90% of the VL cases identified were from the high-incidence stratum, potentially reflecting both the oversampling and higher incidence in the stratum.

**Table 2** Diagnosis of visceral leishmaniasis (VL) among persons attending clinical interview by incidence stratum

| Incidence stratum | VL number (%) | Non-VL number (%) | Total |
|---|---|---|---|
| High | 207 (62.9) | 122 (37.1) | 329 (100) |
| Medium | 18 (25.4) | 53 (74.7) | 71 (100) |
| Low | 2 (4.0) | 48 (96.0) | 50 (100) |
| Total | 227 (50.4) | 223 (49.6) | 450 |

The 227 VL cases resided in 194 households (Table 3). Fifty-eight (13%) occurred in households with > 1 VL case in the past 12 months. Cases were identified in all survey blocks except Narkatia (low incidence). The three blocks in the high-incidence stratum contributed similar numbers of cases.

VL cases ranged in age from 3–70 years: 35% were 10 years or younger and 45% were under 18 years. Men comprised 56%. The most common caste category among the cases was 'Other Backward Castes', while 'Scheduled Castes' were the second most common (28%). Seventy per cent of the houses of cases were of thatch and/or grass, which is characteristic of the poorest households.

The gender distribution of VL cases by status of illness is presented in Table 5. Among cases, the ratio of men to women was 1.3:1. Among the 64 current cases, 32 were men (50%), for a male-to-female ratio of 1:1. In the small group of individuals who died from VL ($n$ = 14), five (36%) were men, yielding a male-to-female ratio of 0.6:1.

### Estimated incidence rates

The estimated weighted annual incidence rate for the high-incidence blocks is 35.6 cases per 10 000, 90% CI: (27.7–45.7). The corresponding estimate for the medium inci-

**Table 3** Number of visceral leishmaniasis (VL) cases per household

| Number of VL cases in household | Number of households (%) | Number of VL cases (%) |
|---|---|---|
| 1 | 169 (87.1) | 169 (74.5) |
| 2 | 19 (9.8) | 38 (16.7) |
| 3 | 4 (2.1) | 12 (5.3) |
| 4 | 2 (1.0) | 8 (3.5) |
| Total | 194 (100) | 227 (100) |

**Table 4** Crude (unweighted) incidence rates of visceral leishmaniasis by block, incidence stratum, and gender

| | Male | | | Female | | | All | | |
|---|---|---|---|---|---|---|---|---|---|
| Incidence stratum | Sample size | Cases | Cases per 10 000 | Sample size | Cases | Cases per 10 000 | Sample size | Cases | Cases per 10 000 |
| High | 34 228 | 113 | 33.0 | 34 352 | 94 | 27.4 | 68 560 | 207 | 30.2 |
| Medium | 6333 | 12 | 18.9 | 6311 | 6 | 9.5 | 12 644 | 18 | 14.2 |
| Low | 4974 | 2 | 4.0 | 4811 | 0 | 0 | 9785 | 2 | 2.0 |
| Total | 45 535 | 127 | 27.9 | 45 574 | 100 | 22.0 | 91 009 | 227 | 24.9 |

**Table 5** Visceral leishmaniasis (VL) case status by gender

| | Male | Female | Total |
|---|---|---|---|
| VL status | Number (Row %) (Column %) | Number (Row %) (Column %) | Number (Row %) (Column %) |
| VL in past 12 months | 90 (60.4) (70.9) | 59 (39.6) (59.0) | 149 (100.0) (65.6) |
| Current VL | 32 (50.0) (25.2) | 32 (50.0) (32.0) | 64 (100.0) (14.2) |
| Death from VL | 5 (35.7) (3.9) | 9 (64.3) (9.0) | 14 (100.0) (3.1) |
| Total cases | 127 (56.0) (100.0) | 100 (44.0) (100.0) | 227 (100.0) |

dence blocks is 16.8 cases per 10 000, 90% CI: (9.3–30.6). With only two cases of VL identified in the low-incidence blocks, no reliable estimate could be made. The weighted estimate of incidence for the high and medium blocks combined is 21.9 cases per 10 000 per year, 90% CI: (14.0–34.2) (Table 6).

## Discussion

The significant strength of this study is the sample, which included more than 5% of the total rural population of East Champaran and 35% of the population of high-incidence areas. The random selection of blocks and villages allows for generalization of the findings to the rural population of the most affected areas of the district. A number of study limitations should be considered.

Prospective active case-finding provides the best estimate of VL incidence. The retrospective survey method used in this study has some limitations. Cases may have been missed if respondents forgot or chose not to report them. There may have been cases among the 21 individuals referred for the clinical interview who did not show up. The severity of VL and the economic burden it imposes leads us to believe diagnosed cases are unlikely to be forgotten. As we encountered no stigma associated with VL, there was no apparent reason not to report a known case.

Of the 450 possible VL cases identified in the household screening, twenty individuals had died in the previous 12 months. Of these, 14 were reported as deaths from VL. It is not known if any of the remaining six were associated with undiagnosed VL. It also is possible that deaths in the past year were underreported during the screening for cultural or personal reasons.

For individuals who could not be examined and lacked medical records, diagnosis could not be made with certainty. Following the death of a family member, the practice in Bihar is to burn medical records. For those individuals absent from the interview and without medical records, VL status was based on the informant's description of symptoms, diagnostic tests, diagnosis, and treatments. This group included the 14 individuals who died from VL and another 23 absent from the clinical

**Table 6** Estimated weighted incidence rates of visceral leishmaniasis (per year per 10 000 persons) for the rural population of East Champaran by incidence strata

| Incidence stratum | Males | Females (90% CI) | All (90% CI) |
|---|---|---|---|
| High | 34.3 (26.9–43.7) | 37.0 (26.9–50.9) | 35.6 (27.7–45.7) |
| Medium | 22.5 (15.3–33.1) | 10.6 (2.2–51.6) | 16.8 (9.3–30.6) |
| Combined high and low | 25.7 (17.7–37.3) | 17.7 (7.5–41.8) | 21.9 (14.0–34.2) |

*The rate for the low-prevalence stratum is not presented because the two cases found there do not permit accurate estimation.

interview. As respondents knew of the interviewers' interest in VL, some may have assumed a potential benefit from reporting a VL diagnosis for the absent person; this assumption could result in an overestimate of VL incidence.

Screening interviews were not obtained in the 6% of selected households vacant at the time of the visit. This is a very low non-response rate, but leaves the possibility of bias, if the absent residents were more or less likely to have VL than those of other households. Anecdotal information from neighbours suggested that villagers were absent more often in search of employment or visiting family than because of illness.

We estimated VL occurrence for a period of one year prior to interview. In a retrospective study, there is uncertainty as to the start of illness. VL symptoms commonly extend for several months prior to diagnosis and treatment. Symptoms with onset prior to the 12 - month window might have been reported as having started within the window (telescoping). Some respondents may have reported the onset of symptoms, while others, the time of diagnosis. Confirmation of the date of diagnosis was only possible when medical records were available. Finally, there also was no assessment of whether the survey case was a new case or relapse.

Our sampling methods based block stratification on government reports of VL cases as these are the only block, district, and statewide data available. While prior research (Singh *et al.* 2006, 2010) suggests that official data underreport cases, for the purposes of this study, we made the assumption that the rate of underreporting does not vary greatly across blocks within a district. Our findings support the use of the government figures in regard to the relative VL case burden across blocks.

The analysis pooled data from two survey rounds in the high-incidence stratum. Each round was independent in that different villages were sampled in each. However, the rounds ascertained cases from two overlapping periods of one year each (Round 1: January–December, 2006; Round 2: May 2006–April 2007. The overlap was 8 months. To test for potential bias from pooling, we compared the estimated rates of VL in the two rounds, in the high-incidence stratum. The estimated rate in Round 1 was 30.1 cases per 10 000 population (95% CI 18.0–50.2); the rate in Round 2 was 38.0 cases per 10 000 (95% C.I. 26.7–53.9). The *P*-value for the difference is $P = 0.45$. We conclude that the bias, if any, from pooling is small.

Another potential source of error was the use of household counts projected from the 2001 census to estimate the household selection probability in each village. In 7 villages, the accuracy of this estimate could be assessed because all households were listed. The average percentage error of the advance estimates was small overall (< ±5%) but there were a few differences larger than ± 20%. Such errors are unlikely to cause much bias, especially as the data were reweighted to reflect the gender and village size distributions in the 2001 census.

As we found only two VL cases among the 9785 residents in households surveyed in the low-incidence stratum, the weighted VL incidence rate for the stratum could not be determined with even moderate accuracy. This stratum includes over 50% of the district's rural population. A reliable estimate of the incidence rate in the district's lower-incidence areas would require a sample at least 20 times as large as ours.

VL cases included those who died in the previous year. Ideally, the denominator for the rate computations would have included person-year contributions from all household members at risk during that time. However, the denominator was based on current counts only, thus it excluded all residents who left the household for any reason, including death, and included current residents who did not live in the household the entire year. Because VL is a rare disease, the discrepancy between current counts and total person- years at risk is unlikely to have caused perceptible bias in the rate estimates.

The finding that one of every eight households with an individual suffering from VL in the past year had more than one VL case during that period indicates the high burden that the disease presents for affected rural households. It also confirms the need for more targeted case-finding in those villages and households from which cases are reported.

Incidence estimates from the blocks reporting moderate numbers of cases at government facilities indicate clearly that the elimination efforts will also have to be directed to those areas. Based on the survey estimate from the two medium-incidence blocks of East Champaran, the estimated annual incidence of VL in the rural areas of those blocks was 16.8 cases per 10 000 population, 90% confidence interval (9.3–30.6), a figure substantially above the elimination program goal.

Prior studies reporting the demographic characteristics of VL cases in South Asia found a higher proportion of male than female patients (Thakur 2000; Barnett *et al.* 2005; Ranjan *et al.* 2005).Our study suggests that this may reflect differential access to treatment across genders. While the ratio among surviving past VL cases, those who were diagnosed and treated, is 1.5 men: one women, it is one men to one women among current cases, some of whom had not been diagnosed or treated for VL, and 0.6 women: one men in the small sample of those who died

from VL. These differences cannot be explained by the population distribution by gender which is almost 1:1, according to the 2001 census figures. The disproportionate number of women among those who die from VL has been reported in prior studies in India and Bangladesh (Ahluwalia *et al.* 2003; Barnett *et al.* 2005). Further research is needed to confirm the extent to which gender-associated barriers to care may explain the higher proportion of male to female VL patients.. Operations research to develop effective outreach interventions to expand women's access to timely, effective, and affordable VL treatment is critically needed if VL elimination is to be achieved.

The survey-based estimated number of cases in 2006 in the high-incidence stratum (1450) is approximately three times the number of cases reported from facilities in the blocks of that stratum (499). This comparison is limited by the absence of information on the residential block of the 25% of reported cases treated at the district hospital. While our sample survey identified 14 VL deaths in the 12- month study period of seven blocks, official reports indicate 15 VL deaths in the entire district at government facilities in 2006, suggesting that deaths from VL are significantly underreported. Unreported deaths may occur at home or at private clinics and hospitals, or be attributed to other diseases.

The Government of India has affirmed its commitment to the elimination of VL by the year 2015 by implementing the Kala Azar Elimination Program in the endemic districts of Bihar under the National Vector-Borne Disease Program. The findings from this study demonstrate the challenge represented by the regional elimination goal of reducing the incidence to one case of VL per 10 000 population per year. Our survey estimates from the district of East Champaran, one of the pilot districts for the national program, suggest that achieving the national elimination target will require a twenty-two fold reduction in the incidence rate in the affected areas, with a thirty-five fold reduction in the most highly affected areas. The political commitment that has been demonstrated will require ongoing support through policies, programs, and resource allocations commensurate to the challenge.

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# USING E-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

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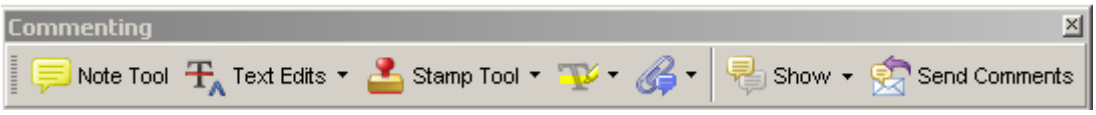
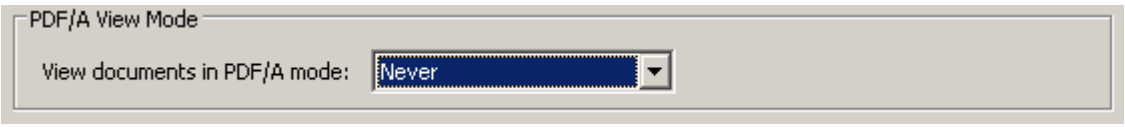


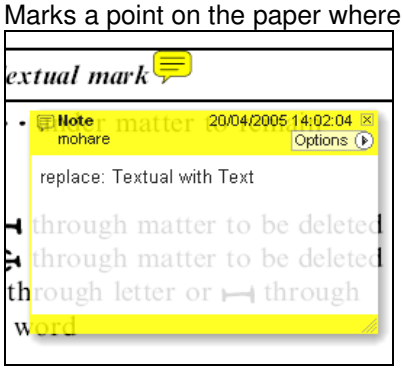

If you experience problems annotating files in Adobe Acrobat Reader 9 then you may need to change a preference setting in order to edit.

In the "Documents" category under "Edit – Preferences", please select the category 'Documents' and change the setting "PDF/A mode:" to "Never".




## Note Tool — For making notes at specific points in the text

Marks a point on the paper where a note or question needs to be addressed.

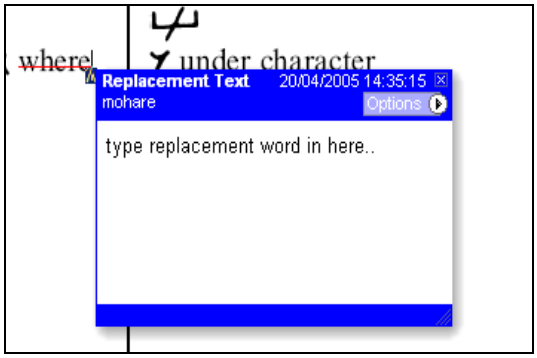



How to use it:

1. Right click into area of either inserted text or relevance to note
2. Select Add Note and a yellow speech bubble symbol and text box will appear
3. Type comment into the text box
4. Click the X in the top right hand corner of the note box to close.

## Replacement text tool — For deleting one word/section of text and replacing it

Strikes red line through text and opens up a replacement text box.

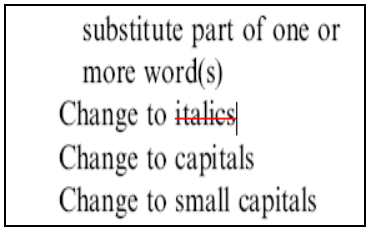



How to use it:

1. Select cursor from toolbar
2. Highlight word or sentence
3. Right click
4. Select Replace Text (Comment) option
5. Type replacement text in blue box
6. Click outside of the blue box to close

## Cross out text tool — For deleting text when there is nothing to replace selection

Strikes through text in a red line.




How to use it:

1. Select cursor from toolbar
2. Highlight word or sentence
3. Right click
4. Select Cross Out Text

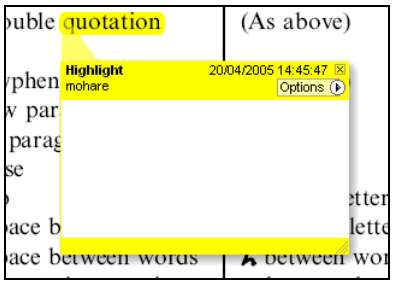
## Approved tool — For approving a proof and that no corrections at all are required.


How to use it:

1. Click on the Stamp Tool in the toolbar
2. Select the Approved rubber stamp from the 'standard business' selection
3. Click on the text where you want to rubber stamp to appear (usually first page)

## Highlight tool — For highlighting selection that should be changed to bold or italic.

Highlights text in yellow and opens up a text box.

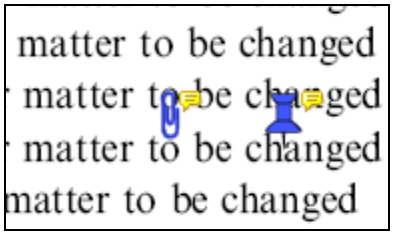



How to use it:

1. Select Highlighter Tool from the commenting toolbar
2. Highlight the desired text
3. Add a note detailing the required change

## Attach File Tool — For inserting large amounts of text or replacement figures as a files.

Inserts symbol and speech bubble where a file has been inserted.

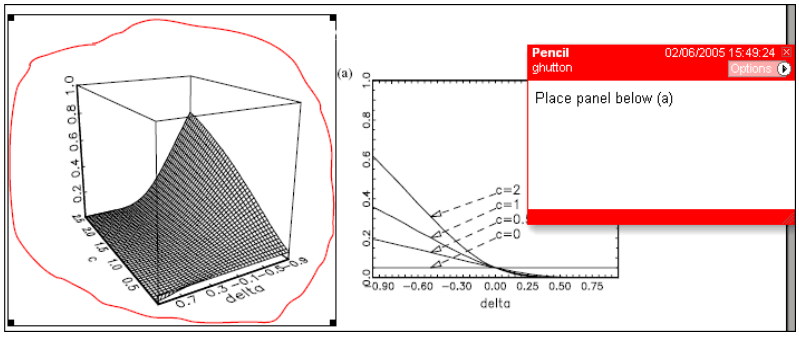



How to use it:

1. Click on paperclip icon in the commenting toolbar
2. Click where you want to insert the attachment
3. Select the saved file from your PC/network
4. Select appearance of icon (paperclip, graph, attachment or tag) and close

## Pencil tool — For circling parts of figures or making freeform marks

Creates freeform shapes with a pencil tool. Particularly with graphics within the proof it may be useful to use the Drawing Markups toolbar. These tools allow you to draw circles, lines and comment on these marks.




How to use it:

1. Select Tools > Drawing Markups > Pencil Tool
2. Draw with the cursor
3. Multiple pieces of pencil annotation can be grouped together
4. Once finished, move the cursor over the shape until an arrowhead appears and right click
5. Select Open Pop-Up Note and type in a details of required change
6. Click the X in the top right hand corner of the note box to close.

## Help

For further information on how to annotate proofs click on the Help button to activate a list of instructions: