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ASYMPTOMATIC INFECTION AND RISK FACTORS FOR LEPTOSPIROSIS IN NICARAGUA

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Abstract. As part of an investigation of a 1995 outbreak of leptospirosis in Nicaragua, a cross-sectional serologic survey was conducted in the town of El Sauce. Of 566 persons, 85 (15%) were positive for IgM anti-*Leptospira* antibodies, indicating recent leptospirosis infection. Asymptomatic leptospirosis infection was common, with only 25 (29.4%) of the 85 seropositive inhabitants reporting a febrile illness in the 2 months before the survey. Multivariable analysis revealed that having an indoor water source remained independently protective against leptospirosis. Gathering wood was independently associated with infection. These findings suggest that asymptomatic infection with *Leptospira* is common in endemic areas of *Leptospira* transmission. Improvement in water sanitation and behavioral modifications to reduce environmental exposure may reduce the risk of leptospirosis in endemic regions.

INTRODUCTION

Leptospirosis is a zoonotic disease caused by spirochetes of the genus *Leptospira*. Humans usually become infected through contact with water or soil contaminated with urine or other body fluids from infected wild or domestic animals. Exposure of skin or mucous membranes to leptospires can lead to infection.[1–3] Clinical signs and symptoms are variable and range from subclinical to potentially fatal manifestations. Subclinical infections have often been reported, and the usually non-specific nature of leptospirosis makes its diagnosis difficult. These mild infections may also be linked to future manifestations of clinical symptoms and chronic disease.[1] The more common mild, anicteric form of the disease is characterized by non-specific symptoms, such as fever, headache, chills, myalgia, nausea, and abdominal pain. The severe, potentially fatal, icteric form of leptospirosis, also known as Weil's syndrome, is typically characterized by renal, hepatic, and vascular complications.[1–3]

In October 1995, following heavy rains which resulted in flooding in the area, 3 patients presented to a village health center in Achuapa, Nicaragua, with acute febrile illness and died within 2 days of acute respiratory distress and hemorrhage. During the next 2 weeks, a sharp increase in unexplained febrile illness was reported in this region, and at least 400 patients were evaluated in the rural health clinics of Achuapa and El Sauce for acute illnesses characterized by fever, headache, chills, and musculoskeletal pain. By early November, 13 of 150 patients who had been referred to the state hospital in the capital city of Leon from the area had died from respiratory distress and pulmonary hemorrhage. In late October 1995, the Nicaraguan Ministry of Health conducted investigations into the outbreak in collaboration with the Centers for Disease Control and Prevention (CDC).[4] Because the clinical presentation being reported was similar to that of dengue hemorrhagic fever, the investigation initially focused around a possible dengue fever epidemic. However, testing of serum samples was negative for dengue virus, hantavirus, and other hemorrhagic viral pathogens. Leptospires were eventually identified through immunohistochemical tests of lung tissue sections from fatal cases.[5]

Following the identification of leptospirosis, several field studies were conducted at Achuapa and El Sauce clinics to describe the extent of the outbreak, identify the epidemic serovars, and identify possible risk factors and prevention strategies.[6] To estimate attack rate and identify predominant risk factors associated with leptospirosis infection during the epidemic, a cross-sectional serologic survey was completed in the town of El Sauce. We report the results of that serologic survey including the epidemic attack rate of leptospirosis in this population, the association between modifiable risk factors and leptospirosis infection, and the prevalence of asymptomatic infection.

METHODS

Study design and time. A cross-sectional survey was carried out in the town of El Sauce, Nicaragua, in early December 1995.

Study site, population, and sample methodology. El Sauce has a population of about 28,000 and is located approximately 125 miles north of Managua. Farming is the primary occupation in the community. Urban and rural households were randomly selected for this study using detailed maps of the town of El Sauce and surrounding townships. If the household members were not present, the next household in the counterclockwise direction was selected.

A standardized questionnaire that included household and individual exposure variables was administered at each household. The head of the household was asked to respond to household questions regarding sanitation system, water source, food source, ownership of animals, and an estimate of rats or rodents in household. In addition, each individual of the household present at the time responded to questions that included demographic information, history of febrile illness, and individual occupational and environmental exposure histories that represented potential risk factors for leptospirosis. Blood samples were collected at the time of the interview and sent to the Centers for Disease Control and Prevention (CDC) laboratory for serologic analysis of leptospiral antibodies. This project was reviewed by the Human Subjects Coordinator in the National Center for Infectious Diseases and determined to be a public health response that did not require IRB review.

Laboratory. Blood specimens were collected, allowed to coagulate for 6 hours at 20°C, aliquotted into freezer tubes, and frozen at −20°C until testing. Within 6 months, the samples were thawed and tested for anti-*Leptospira* IgM antibodies using an IgM anti-leptospiral enzyme-linked immunosorbent assay (ELISA, PanBio, PanBio Inc, Brisbane, Australia) at the CDC. The assays were performed and interpreted according to the manufacturer's instructions.

Case definition. A case was defined a person who participated in the survey and was positive for anti-leptospiral antibodies by IgM ELISA as defined by the manufacturer. Because of the timing of the survey in relation to the peak of the epidemic (approximately 2 months after), and the tendency for IgM antibodies to last an average of 3 to 6 months,[7–9] these were considered recent infections and indicative of incident cases.

Statistical analysis. SAS software system release 6.12 (SAS Institute, Cary, NC) was used to derive descriptive statistics and in subsequent multivariable analyses. All variables were dichotomized except for age, which was categorized based on quartiles of the age distribution in the data set.

Univariate analyses compared infected persons and non-infected persons for demographic, environmental, behavioral, and occupational exposure variables. Logistic regression models were fit using the SAS GENMOD procedure with an exchangeable correlation matrix for both univariate and multivariable analyses. This technique uses generalized estimating equations and accounts for the potential within-household correlation of the data. Significance in univariate analysis was determined with a Wald chi-square P-value ≤ 0.05.

To control for confounding and to determine which exposures were most important in predicting seropositivity, a multivariable model was created containing exposures with a univariate significant P-value ($P \leq 0.05$), potential confounders, and interaction terms. A backward elimination procedure was used to identify significant interaction terms and exposure variables that were most strongly associated with seropositivity for leptospirosis. Variables with Wald P-values ≤ 0.05 were considered to be significant. The 95% confidence limits for the odds ratios that involved the estimated coefficient of any significant interaction term were calculated using the variance-covariance matrix. Multicollinearity among two or more variables was assessed using a diagnostic technique that calculates condition indices and variance decomposition proportions.

RESULTS

Eight hundred and sixty-seven persons from 218 households were enrolled. Blood samples were tested from 566 (65%) of these individuals from 207 households. The remaining 301 (35%) persons declined phlebotomy. Only the 566 persons with blood test results were included in the final analysis. Of the 566 samples tested, 85 were positive for IgM anti-*Leptospira* antibodies, indicating recent leptospiral infection (attack rate = 15%).

Asymptomatic infection. Of the 85 persons who were seropositive by IgM ELISA, only 25 (29.4%) reported having a febrile illness during the preceding 2 months; the remaining 60 (70.6%) had asymptomatic infection.

TABLE 1

Univariate results of demographic risk factors among infected and non-infected persons: odds ratios (OR), 95% confidence intervals (CI), and Wald chi-square P-values

Variable	Infected (n = 85) n (%)	Non-infected (n = 481) n (%)	OR (95% CI)	P
Rural household	28 (32.9)	92 (19.1)	2.1 (1.2–3.6)	0.0085
Sex				
Male	24 (28.2)	170 (35.5)	0.7 (0.5–1.2)	0.1891
Female	61 (71.8)	309 (64.5)	1 (referent)	referent
Age (yr)				
5–13	22 (22.2)	129 (26.9)	1.5 (0.7–3.0)	0.2921
14–22	23 (27.4)	109 (22.7)	1.8 (1.0–3.3)	0.071
23–40	24 (28.6)	119 (24.8)	1.7 (0.9–3.3)	0.1117
>40	15 (17.9)	123 (25.6)	1 (referent)	referent

Descriptive statistics and univariate analysis. The univariate associations between exposures and leptospiral infection in this population sample are presented in Tables 1 through 3.

The median age among the infected persons (cases) was 21 years (range: 5–78) and median age among the non-infected persons was 23 (range: 5–93). Based on quartiles, four age groups were created for univariate analysis: 5–13 years, 14–22 years, 23–40 years, and > 40 years. The highest age group (> 40) had the lowest infection rate and was used as the referent group for the statistical comparisons. None of the age groups was found to be significantly associated with infection ($P > 0.05$) when compared with the referent group (Table 1). The age-specific attack rates are shown in Figure 1.

Among the sample population, 120 (21.2%) lived in rural households and 446 (78.8%) lived in urban households. In univariate analysis, living in a rural household was significantly associated with infection, with 28 (32.9%) of the 85 infected persons and 92 (19.1%) of the 481 non-infected persons living in rural household (OR = 2.08, 95% CI = 1.21, 3.60, $P = 0.0085$). The anti-leptospiral IgM antibody prevalence in the rural population (23.3%) was nearly twice the prevalence in the urban population (12.8%).

Gender was not significantly associated with infection. The infection rate among females (16.5%) was slightly higher than males (12.4%). Females were significantly over-represented in the study sample compared with the general population (65.6% versus 50%, $P \leq 0.05$).

Household factors. A majority of the study population (85.3%) had an outdoor latrine for the household sanitary facility (Table 2). Type of household sanitary facility was not associated with infection.

Several questions were asked about household items that might reflect socioeconomic status. Ownership of a radio or television was more frequent among non-infected than infected persons and was shown to be associated with significant protection in univariate analysis.

Having an indoor household water source was more frequent among non-infected than infected persons (193 [40.1%] versus 15 [17.7%]) and was associated with significant protection (OR = 0.32, 95% CI = 0.18, 0.60, $P = 0.0002$) in univariate analysis. Private well and river/lake/spring water sources were significantly associated with in-

TABLE 2

Univariate results of potential household risk factors among infected and non-infected persons: odds ratios (OR), 95% confidence intervals (CI), and Wald chi-square *P*-values

Variable	Infected (n = 85) n (%)	Non-infected (n = 481) n (%)	OR (95% CI)	*P*
Household sanitary facility				
Outdoor latrine	76 (89.4)	407 (84.6)	1.6 (0.8–3.0)	0.1818
No latrine or plumbing	3 (3.5)	24 (5)	0.7 (0.2–2.0)	0.5109
Indoor plumbing	6 (7.1)	48 (10.0)	0.7 (0.3–1.5)	0.3546
Other	3 (3.5)	8 (1.7)	2.1 (0.7–6.5)	0.2208
Household appliances				
Electric lights	60 (70.6)	376 (78.2)	0.7 (0.4–1.1)	0.1178
Radio	56 (65.9)	369 (76.7)	0.6 (0.4–1.0)	0.0391
Television	40 (47.1)	297 (61.8)	0.6 (0.3–0.9)	0.0252
Refrigerator	11 (12.9)	109 (22.7)	0.5 (0.3–1.0)	0.0568
Motor vehicle	2 (2.4)	24 (5.0)	0.4 (0.1–1.5)	0.1774
Telephone	1 (1.2)	25 (5.2)	0.2 (0.03–1.7)	0.1480
Household water source				
Public facility	9 (10.6)	17 (3.5)	3.2 (1.0–10.2)	0.0516
Private well	8 (9.4)	26 (5.4)	1.9 (1.1–3.5)	0.0333
Public well	13 (15.3)	53 (11.0)	1.5 (0.7–3.0)	0.2746
River or lake	1 (1.2)	4 (0.8)	1.4 (1.1–1.9)	0.0067
Patio	47 (55.3)	250 (52.0)	1.2 (0.7–2.0)	0.5425
Other	13 (15.3)	62 (12.9)	1.2 (0.6–2.1)	0.6311
Buy water	1 (1.2)	8 (1.7)	0.7 (0.1–4.5)	0.6857
Indoor	15 (17.7)	193 (40.1)	0.3 (0.2–0.6)	0.0002
Household animal ownership				
Horse	11 (12.9)	40 (8.3)	1.6 (0.9–2.9)	0.1087
Cow	13 (15.3)	64 (13.3)	1.1 (0.6–2.1)	0.7118
Pig	38 (44.7)	214 (44.5)	1.1 (0.6–1.7)	0.8406
Dog	49 (57.7)	273 (56.8)	1.0 (0.6–1.8)	0.8615
Rodent exposure				
Saw rodents/rats in house in October	73 (90.1)	400 (85.1)	1.5 (0.6–4.0)	0.3646
Avg. times seen per week >3	42 (65.6)	222 (64.7)	1.0 (0.6–2.0)	0.8854

fection in univariate analysis, although the numbers included in these categories were relatively low.

Exposure to rodents was assessed by asking if rats or rodents were seen in the house in the month of October, the month prior to the outbreak of leptospirosis. Exposure categories were assessed as any versus none, and high ($>$ 3 times per week) versus low ($\leq$ 3 times per week). A high proportion (85.8%) of the study population reported having seen rats or rodents in their houses. There was no difference between infected and non-infected persons with either of the rodent exposure variables.

Individual activities. In univariate analysis, infected persons were significantly more likely to have gathered wood ($P = 0.0003$), ground grain ($P = 0.0011$), shelled or husked corn ($P = 0.0276$), washed clothes in a river or brook ($P = 0.0104$), or taken a bath in a river or brook ($P = 0.0039$) during the months of October or November (Table 3).

Multivariable analysis. Age (3 indicator variables) and sex were retained in the model throughout the backward elimination procedure to control for any confounding effect. Multivariable backward logistic regression analysis revealed that having an indoor water source ($OR_{adjusted} = 0.42$, 95% CI = 0.22, 0.80) remained independently associated with protection against leptospirosis, and gathering wood was independently associated with infection ($OR_{adjusted} = 2.08$, 95% CI = 1.14, 3.79) (Table 4).

The only interaction term that remained significant in the model was interaction between living in a rural household and shelling or husking corn ($P = 0.010$). The interaction in the multivariable logistic model suggested that husking or shelling corn was positively associated with infection among urban residents ($OR_{adjusted} = 1.8$, 95% CI = 0.72, 4.51) and protective among the rural residents ($OR_{adjusted} = 0.32$, 95% CI = 0.04, 2.27), although neither odds ratio was significant. No multicollinearity among the variables was detected.

DISCUSSION

Leptospirosis is usually characterized as a febrile illness. Subclinical or asymptomatic leptospirosis is not well understood. Previous clinical reports have indicated that fever is present in most[11] or all cases.[12] In this study, only 29.4% of those with evidence of leptospirosis infection reported a febrile illness during the preceding 2 months. The remaining seropositive individuals were considered to have had asymptomatic leptospiral infection. This finding, along with other reports of afebrile leptospiral infection,[13,14] suggests that fever may be more frequently absent or mild in leptospirosis infection than previously described.

Asymptomatic infection has been reported in other studies.[11,15–17] In a study in the Seychelles, 9% of adult males had laboratory results consistent with recent leptospiral infection, and 37% had evidence of past leptospiral infection, with no one reporting current or past symptoms related to

TABLE 3

Frequency of potential individual risk factors among infected and non-infected persons: odds ratios (OR), 95% confidence intervals (CI), and Wald chi-square *P*-values

Activity	Infected (n = 85) n (%)	Non-infected (n = 481) n (%)	OR (95% CI)	*P*
Individual activities				
Take bath in river or brook	23 (27.1)	65 (13.5)	2.5 (1.3–4.6)	0.0039
Gather water at river or brook	1 (1.2)	2 (0.4)	2.5 (0.4–13.9)	0.3011
Wash clothes in river or brook	23 (27.1)	63 (13.1)	2.4 (1.2–4.7)	0.0104
Swim in river or brook	11 (12.9)	47 (9.8)	1.5 (0.7–3.2)	0.2432
Gather wood	45 (52.9)	150 (31.2)	2.4 (1.5–3.9)	0.0003
Grind grain	32 (37.7)	99 (20.6)	2.3 (1.4–3.8)	0.0011
Shell/husk corn	30 (35.3)	108 (22.5)	1.9 (1.1–3.2)	0.0276
Walk in mud	5 (5.9)	18 (3.8)	1.8 (0.6–4.9)	0.2676
Pasture cattle/livestock	15 (17.7)	57 (11.9)	1.6 (0.9–2.8)	0.0967
Cook	56 (65.9)	265 (55.1)	1.5 (0.9–2.4)	0.1084
Walk across brooks	18 (21.2)	71 (14.8)	1.5 (0.8–3.1)	0.2237
Work in the field	24 (28.2)	96 (20.0)	1.5 (0.8–2.7)	0.1647
Clean house	64 (75.3)	329 (68.4)	1.3 (0.8–2.2)	0.2480
Walk barefoot or with sandals out of the house	47 (55.3)	234 (48.7)	1.3 (0.8–2.1)	0.3323
Travel out of community in October	19 (22.4)	83 (17.3)	1.3 (0.7–2.5)	0.3407
Wash clothes in house	53 (62.4)	303 (63.0)	1.0 (0.6–1.6)	0.9068

leptospirosis.[15] In another study conducted in Brazil, 22% of study subjects were positive for IgM antibodies but were asymptomatic.[16] In a seroprevalence study of leptospirosis in Bangladesh, among serum samples from 31 individuals without a history of clinical illness and originally selected to serve as controls, 15 (48%) were seropositive for leptospiral infection.[17] Leptospiral antibodies in asymptomatic individuals may provide immunity against leptospirosis, especially in areas of endemic disease. Because person-to-person transmission of this disease is rare,[1,18] it is unlikely that asymptomatic infected individuals are important in the transmission of leptospirosis.

The main objectives of this study were to determine the attack rate and identify risk factors associated with leptospirosis in the town of El Sauce, Nicaragua, following an outbreak that occurred after heavy rains and flooding.[6] Based on this serosurvey, we found that the outbreak attack rate in the population was 15% as determined by IgM ELISA. IgM antibodies against leptospires develop approximately 4 to 6 days after exposure.[3] The persistence of these IgM antibodies is unknown,[3] but some studies report that IgM antibodies persist 3 to 6 months past the onset of illness.[7–9] We conducted this serosurvey 2 months following the peak of the outbreak in October of 1995. Therefore we can assume that the estimated attack rate determined in this study was an accurate reflection of the true attack rate.

Among those infected persons, 71.8% were female and 28.2% were male. This difference reflects the overrepresentation of females in the study population (65.6% females versus 34.4% males). In Nicaragua, females tend to work in the home, and therefore were more likely to be present at the time of the survey.

Living in a rural household was significantly associated with infection in univariate analysis. The higher seroprevalence in the rural area is consistent with other surveys[13,19,20] and may be attributed to the different activities and lifestyle of rural versus urban populations. Occupationally-related activities such as gathering wood, grinding grain, and shelling/husking corn were also significantly associated with infection in univariate analysis. Exposure to leptospires in these cases may be a result of environmental exposure to contaminated surfaces, mud, or infected animals during these activities. Persons who are involved in farming and other agricultural occupations are at higher risk for disease because of exposure to contaminated water, mud, and urine.[1] Other occupationally-related activities assessed in the study were reported more frequently among infected than non-infected persons, such as working in the field or pasturing livestock, but the association was not significant.

Behavioral factors significantly associated with infection in univariate analysis included washing clothes or bathing in a river or brook. In our concurrent case-control study conducted in the neighboring towns of Achuapa and El Sauce, walking through creeks or swimming in rivers were significant risk factors, and exposure to contaminated flood waters was found to be a likely cause of the epidemic.[6]

In this serosurvey, in univariate analysis, ownership of a household radio or television was significantly protective against leptospirosis. This finding may indicate that those in

TABLE 4

Risk factors for leptospiral infection by multivariable logistic regression adjusted for age and sex

Variable	Adjusted OR (95% CI)	*P*
Rural household†	2.61 (1.06–6.45)	0.03737
Gathering wood	2.08 (1.14–3.79)	0.01717
Shelling/husking corn*†	1.8 (0.72–4.51)	0.21125
Indoor water source	0.42 (0.22–0.80)	0.00902
Rural household × shelling/husking corn	NA	0.01014

OR = Odds Ratio; CI = Confidence Interval; NA = Not applicable.

* Stratification on rural and urban households was done to analyze the effect of shelling or husking corn on leptospiral infection because significant interaction was present in the final multivariable model. The stratum specific odds ratios and confidence intervals for the effect of shelling or husking corn are listed as follows:

Shelling or Husking Corn among the urban: OR = 1.80, 95% CI = (0.72, 4.51)

Shelling or Husking Corn among the rural: OR = 0.32, 95% CI = (0.04, 2.27).

† Variables for rural household and shelling or husking corn were retained in the model throughout the backward elimination procedure of the exposure variables because these variables were lower-order components of the significant interaction term (Hierarchically Well Formulated Model Principle).[10] Otherwise both variables would have been removed because of nonsignificance earlier in the procedure.

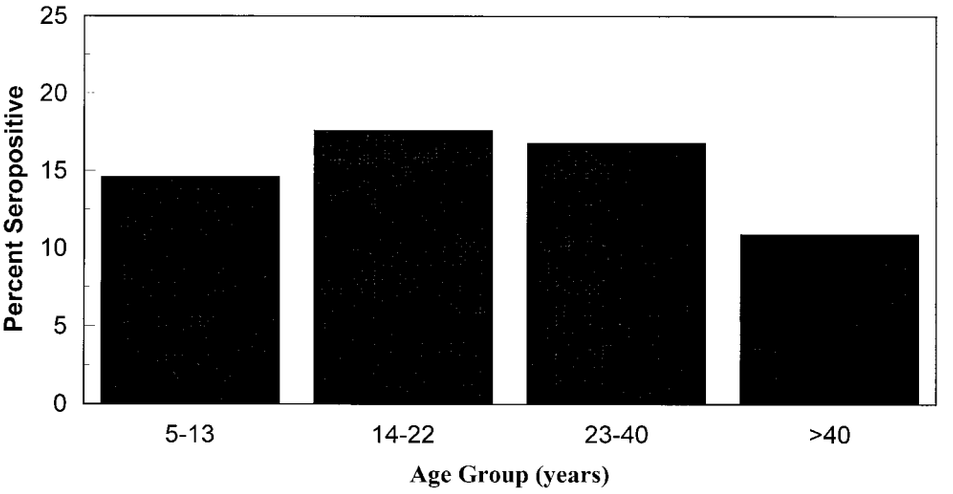

FIGURE 1. Age-specific attack rate for leptospirosis infection in El Sauce, Nicaragua, 1995.

a lower socioeconomic status are more likely to be exposed to *Leptospira.*

In the multivariable logistic model, having an indoor water source ($OR_{adjusted}$ = 0.42, 95% CI = 0.22, 0.80) remained independently associated with protection against leptospiral infection and gathering wood was independently associated with infection ($OR_{adjusted}$ = 2.08, 95% CI = 1.14, 3.79), suggesting that these two factors were the strongest independent predictors. Our finding that an indoor water source protects against infection, together with other studies implicating the ingestion of water from contaminated sources as a significant risk factor,[21–23] emphasizes the importance of water sanitation and treatment in the prevention of human leptospirosis. The association with gathering wood may represent a marker for other outdoor activities that bring people into contact with contaminated water, or may reflect contact with animal urine. People who gather wood may also be more likely to have cuts and broken skin, which may provide a route for leptospires to enter the body. In a previous case control study in the Seychelles, leptospirosis was positively associated with activities in forests, which may be related to an increase in exposure to the environment.[15] The exact explanation for the association with gathering wood requires further investigation.

The significant interaction term between rural household and shelling and husking corn was left in the multivariable model because living in a rural or urban household significantly modified the independent effect of shelling or husking corn on leptospiral infection. This suggests that location of residence influenced the association of shelling or husking corn with infection.

The results of the previously reported case-control study investigating the Nicaragua outbreak suggested that peridomestic amplification might have been important in leptospiral transmission. A number of factors in that study demonstrated that dogs played a major role in environmental contamination during the epidemic.[6] Although none of the domestic animal exposures in this serosurvey were found to be associated with infection, the activities that were significant suggest that indirect exposure to animal urine did contribute to disease transmission. Although exposure to rodents was not significantly associated with infection, this might have been due to the fact that a high proportion of both infected and non-infected individuals reported seeing rodents in their houses during the month of October.

Our data suggest that overt illness may be present in a minority of all leptospirosis infections. While the importance of asymptomatic infections in the ecology of leptospirosis remains to be defined, these asymptomatic infections may contribute to herd immunity. Findings from this serologic survey suggest that improvements in water source and behavioral modifications to reduce environmental exposure may reduce the risk of leptospiral infection in this region.

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