Impacts of human activities and illegal nonhuman primate trade on yellow fever epidemiology in Colombia – A pilot project

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Summary
Yellow fever is endemic to 30-40% of Colombia's territory, affecting human and nonhuman primate health and having great economic impact on affected communities. Most of the studies conducted so far in the country on yellow fever epidemiology have focused on determining the species of vectors and vertebrate host involved in the disease transmission cycle and the effects on human populations. But little has been done to evaluate the dynamic interactions of these factors at the interface of human, animal and environmental health. Using a multidisciplinary One-Health approach, this study sought to identify yellow fever risk factors for human communities and nonhuman primate populations in Colombia. We incorporated methodologies such as ethnoprimatology and participatory epidemiology coupled with epidemiological tools such as serology to facilitate the assessment of a possible causal relationship of occupational activities, degree of contact with nonhuman primates, and nonhuman primate trade with the risk of being exposed to yellow fever infection. Serology for yellow fever antibodies was negative in the captive population of howler monkeys tested. The occupational activities, degree of interaction with nonhuman primates and presence of nonhuman primate trade were determined by conducting observations, focus group meetings, and individual questionnaires with different communities located at various distances from the captive and free-ranging nonhuman primate populations. Although the entire analysis of the qualitative data has not been completed, so far no evidence of nonhuman primate or wildlife trade has been found in the study area. We identified as the main occupational activities agriculture, livestock production, and housekeeping; and the data suggest that the degree of interaction between humans and nonhuman primate varied depending upon the activities conducted and/or their household proximity to nonhuman primate populations' territory. Further analysis is required to determine if there is a correlation between human activities and known yellow fever risk factors.

Background
Yellow fever (YF) is a zoonotic, mosquito-borne viral hemorrhagic disease endemic to tropical areas of South America and Africa[4,9]. It is estimated that each year approximately five million people are at risk of YF infection in Colombia[6,11,12]. It affects humans and wildlife. Howler monkeys are very suceptible and often present high mortality rates when infected. Colombia has 10 recognized endemic areas for yellow fever, which represents 30-40% of the country's territory. These areas are characterized by forested areas near river banks where small outbreaks often occur[6,8,11]. The rest are considered free or epidemic-areas.

Disease incidence and dispersal depends upon several factors such as human exposure (related to human behavior and occupational activities – e.g. logging, hunting, mining, ecotourism), environmental factors, vertebrate host susceptibility, vector characteristics and ecological patterns[2,7,10]. A clear example of this was the 2003 outbreak, when primate epizootics and a total of 104 cases were reported, with 41 (39.5%) fatal cases, after 4 months. This was one of the largest outbreaks documented over the last 50 years[11]. The rapidly propagation was associated with the movement of infected people into non-endemic areas and the suceptibility of the communities. This outbreak served to show one of the first clear connections between Colombia's social factors and yellow fever disease epidemiology. There was evidence that pointed to the harvesting of illicit crops (paired with the movement of susceptible people to the area) as the culprit for the outbreak and disease dispersal[11,12].

Using a multidisciplinary One–health approach these social factors, as well as other drivers could be more acurately evaluated and associated with YF disease transmission. It could also facilitate the design and evaluation of disease surveillance and management strategies. These have been accomplished for the control of diseases in Africa and Asia (e.g. rinderpest erradication and avian influenza surveillance) through the incorporation of participatory epidemiology in conjuction with epidemiological tools such as serology[1,3,5].

Our research interests are to understand the yellow fever risk factors in Colombia resulting from human activities, behaviors and types of interaction with NHPs (nonhuman primates) through illegal trade, hunting or by sharing the same environments. Our hope is to facilitate future design of an enhanced surveillance program for yellow fever in Colombia that takes social realities and epizootic (nonhuman primate yellow fever cases) surveillance into account.

Goals

In the initial proposal of the project the goals set were more broad and focused mainly in the evaluation of the presence of antibodies for YF in howler monkeys confiscated from the illegal wildlife trade and free-ranging. However, due to limited time and constraints with permits in Colombia these goals were modified to allow for concrete data gathering. This evolution of purpose resulted in the following goals:

- Obtain training in YF surveillance and howler monkey trapping from collaborating researchers in Rio Grande do Sul, Brazil.

- Evaluate the presence of antibodies to YF in howler monkeys confiscated from the illegal wildlife trade.

- Identify local occupational activities, type of interaction with NHP, perception and knowledge of zoonotic diseases, current surveillance, and NHP conservation efforts in the area in order to determine the community's risk to YF infection.

Study area

Brazil

The training was conducted with collaborators at the Centro Estadual de Vigilância em Saúde (CEVS), Rio Grande do Sul, Brazil. The field trip to capture howler monkeys and sample for yellow fever was conducted in the Morro São Pedro in the South of Porto Alegre. This area is known for the presence of free-ranging howler monkey populations and is well preserved.

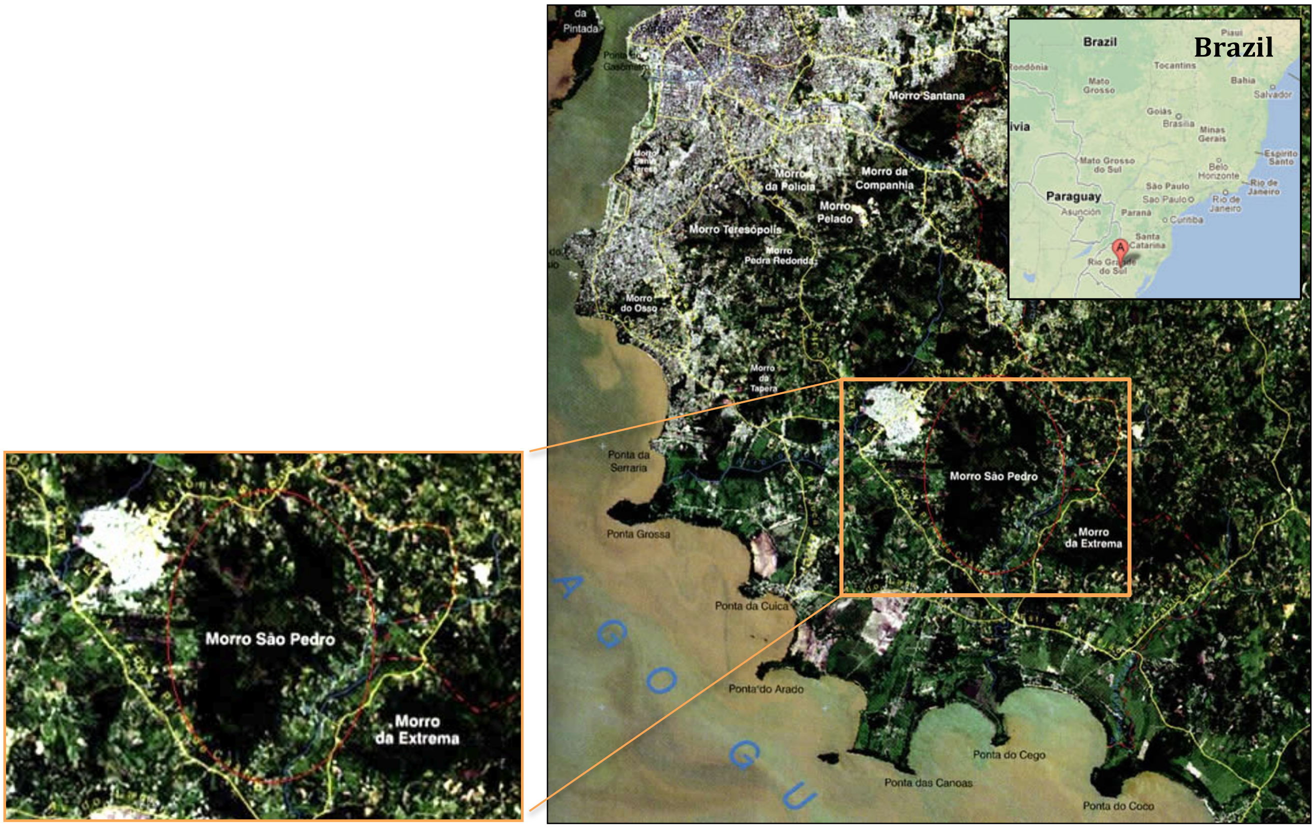

Figure 1: Study area map for free-ranging howler monkey trapping, Morro São Pedro, Rio Grande do Sul, Brazil.

Colombia

The study area selected for conducting the seroprevalence for YF antibodies was the Ecosantafe rehabilitation center for howler monkeys (Fundacion Ecosantafe), part of the SantaFe Zoo. The center was located in the community of La Cascada, municipality of Jerico, Antioquia in northeastern Colombia. The center is recognized nationaly for their howler monkey rehabilitation program. This is the only organization in the Country permitted to admit howler monkeys confiscated by environmental agencies from the illegal wildlife trade. The center is characterized for being located at 1200 mamsl, a warm-temperate climate, and for its close proximity to free-ranging howler monkey populations.

The participatory assessment component of the research project was conducted at the municipality of Jerico, Tarso and Pueblorrico, Antioquia. The area was selected based on their proximity to the location where the blood samples from confiscated howler monkeys was taken to evaluate for yellow fever antibodies. Also because it is an area characterized by the presence of free-ranging nonhuman primate populations and an area considered free of yellow fever but situated close to a recognized endemic yellow fever area in the country.

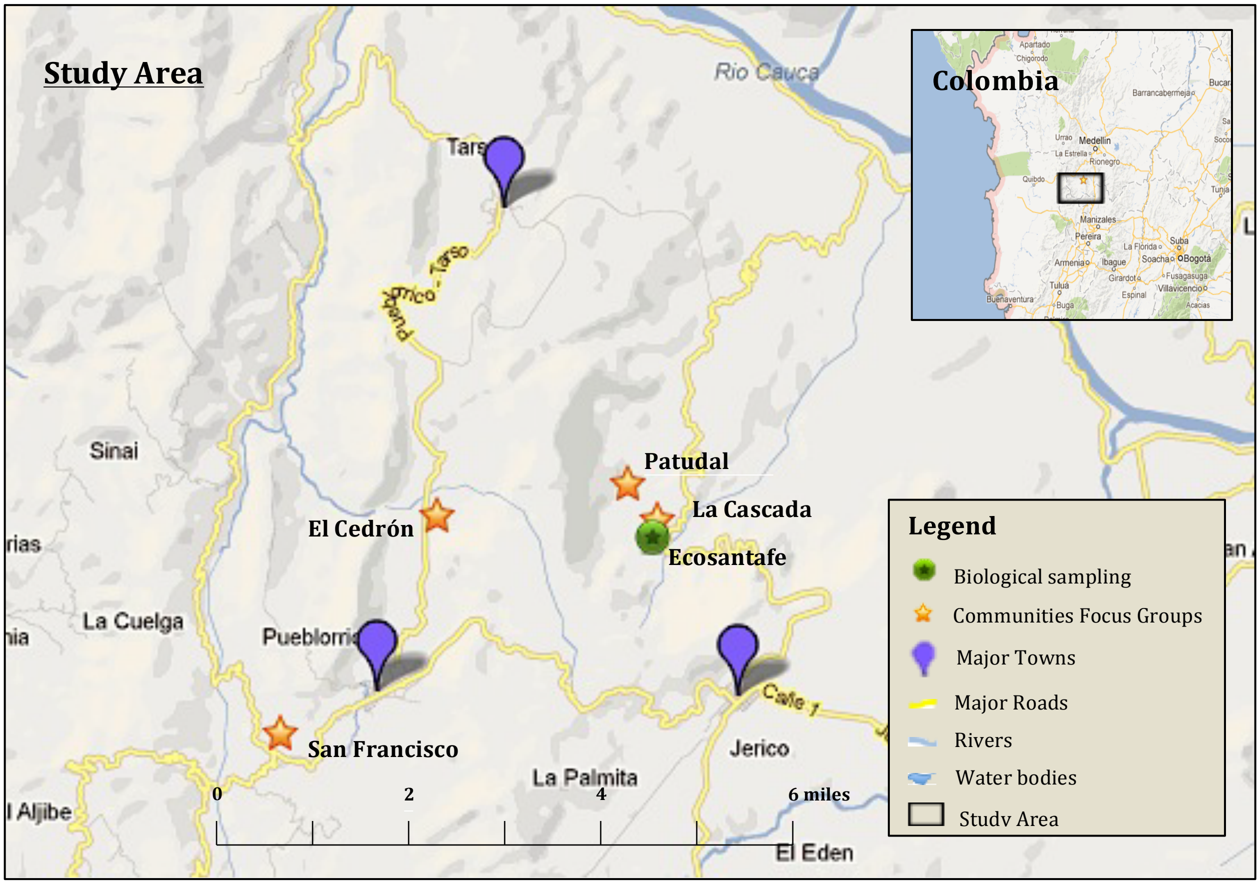

Figure 2: Study area map (Antioquia, Colombia)

Study species

Initially we chose howler monkeys and capuchins monkeys are the species for evaluating the presence of antibodies against yellow fever. However, due to permits and time constrictions we only conducted biological sampling in howler monkeys.

Howler monkeys are widely distributed across South America and Colombia. The species was chosen based on evidence provided by studies conducted in Brazil by the Ministry of Health and in French Guiana by other researchers that shows that howler monkeys play an important role in the dynamics of YF in the Americas and that they can serve as good sentinels for the disease. This is due to the species' high susceptibly to YF, its wide geographic distribution in South America, including Colombia, and its presence in Colombia's yellow fever endemic areas.

Human subject population

The subject population for this project included a wide net of individuals from multiple social strata, gender, occupation, and adult age groups. The diversity of participants allowed us to gather more representative data and local knowledge.

- Inclusion criteria: (1) Lived in study area more than 50% of the year, (2) Adults (age 18 and older) both male and female mentaly competent, (3) chose to come to the focus group or questionnaire session, (4) consented to participate.

- Exclusion criteria: (1) Lived in the study area less than 50% of the year, (2) less than 18 years of age or mentaly incompent, (3) chose not to come to the focus group or questionnaire session, (4) did not consent to participate.

- Withdrawal/Termination criteria: Individual was aggressive, excessively overpowering, or chose to cease participation for any reason. This came into play only if the safety of other participants was threatened.

2012 Accomplishments – Brazil

Training on Yellow fever surveillance - I spent a total of 3 weeks with collaborators at the Centro Estadual de Vigilância em Saúde (CEVS), Rio Grande do Sul, Brazil, where I learned about their intensive active YF epizootic surveillance program implemented in 2001. Most of these were done by one-on-one conversations with the researchers, as well as through evaluation of field notes, papers and raw data. As part of the training of YF surveillance I had a demonstration of the different techniques and equipment used for mosquito sampling.

Training on Howler monkey trapping - During my time at CEVS I had the opportunity to observe and assist with the capture of free-ranging howler monkeys during a field expedition conducted at a well-preserved conservation area south of Porto Alegre city called Morro São Pedro. The field expedition was conducted as part of their epizootic yellow fever surveillance and it allowed me to understand the best methods for capturing howler monkeys, including the required supplies and personnel. A total of three individuals were captured using CO_2 dart-guns and nets to cushion the fall, then processed for yellow fever samples, clinically evaluated, and marked before being released back into the wild.

Figures 3-6: Sequence of photos from capture and biological sampling (Morro São Pedro, RS, Brazil)

Promotion of the application of the Conservation Medicine/One Health discipline – As a foreigner researcher I had the opportunity to promote application of Conservation Medicine/One Health discipline to biology students involved in the study of nonhuman primates, and to public health veterinarians at CEVS. This was done through several presentations about my case study in yellow fever epidemiology in Colombia and my fellowship research proposal.

2013 Accomplishments – Colombia

Biological sampling of confiscated howler monkeys – A total of 16 confiscated howler monkeys, including 10 males and 6 females, were evaluated at the Ecosantafe rehabilitation center. All of them had a full physical exam, morphometric measurements taken, blood sample, feces and ectoparasites collected. All 16 animals were from different ages including juveniles, sub-adults and adults. The blood and fecal samples and blood smears were analyzed at the Clinical Pathology Laboratory at the Universidad de Antioquia in Medellin and the serum collected was analyzed for yellow fever antibodies at the National Institute of Health in Bogota, Colombia.

Figure 7. Physical examination

Figure 8. Biological sampling

Figure 9. (a) Blood centrifugation and (b) serum separation

Human activities and illegal nonhuman trade participatory assessment – The participatory assessment was conducted in four communities located at the municipalities of Jerico, Tarso and Pueblorrico. The four communities were Patudal and La Cascada located in Jerico; El Cedron located in Tarso; and San Francisco located in Pueblorrico. All of the communities were selected randomly and were located between a 10-mile radius around the rehabilitation center where the biological samples were collected. In each community 5-20 individual questionnaires, 1 focus group meeting and two transect walk observations were conducted. A total of 72 persons including both sexes participated from the study, 40 participated from the focus group meetings and 32 from the individual questionnaires.

Figure 10a-b. Observations (Transect walks through the communities)

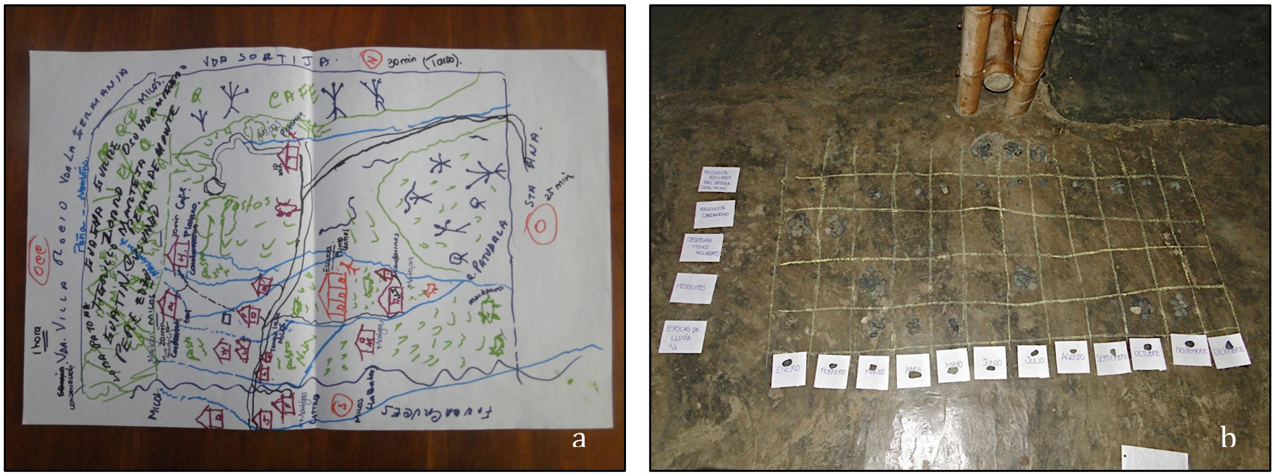

Figure 11. Focus groups (a) Community mapping and (b) Seasonal calendar

Results

Brazil

Having first hand experience with the researchers allowed me to get familiarized with the challenges, and the pros and cons of carry on an intensive epizootic surveillance program for YF. I also gained knowledge on howler monkey trapping and mosquito sampling, and became familiar with the different techniques, equipment and personnel required for conducting a successful surveillance study.

Biological sample results – The sample results collected from the three howler monkeys at Morro São Pedro were all negative for yellow fever and dengue antibodies through viral isolation using C6/36 cells, immunofluorescence and complement fixation. Also the samples were negative by inhibition of hemagglutination for 19 arboviruses tested (Alphavirus: EEE, WEE, Mayaro, and Mucambo; Phlebovirus: Icoaraci; Orthonunyavirus: Guaroa, Maguari. Tacaiuma, Uringa, Belem, Capaparu, Oropuche, and Catu; Flavivirus: Yellow fever, Ilhaus, St. Louis, Cacicapore, Bussuquara, and Roci).

Colombia

The data collected during the pilot project is still in the process of analysis. Results for the biological sampling are described below, however, the results of the participatory assessment are still in the initial phases of qualitative analysis. Complete results of this project will be available for TIE when the analysis of the qualitative data is completed and published.

Biological sampling of confiscated howler monkeys - A total of 16 individuals were restrained and sampled for determining yellow fever antibodies seroprevalence. On physical exam all animals were healthy. However, some minor lesions and signs of diseases were found on the population, those included broken or missing teeth, periodontal disease, mal-occlusion, old resolved fractures, signs of metabolic bone disease already corrected, bite wounds and lacerations, dermatitis, alopecia and stress diarrhea. No ectoparasites and hemoparasites were found in the animals and only one fecal sample was positive for trophozoites of *Chilomastix mesnili.*
All 16-serum samples collected from the population were negative for yellow fever antibodies by capture ELISA and inhibition of hemagglutination (HI).

Human activities and illegal nonhuman trade participatory assessment – A total of four communities were included in the participatory assessment. Two of them Patudal y La Cascada (Jerico municipality were located between 0-3 miles from the rehabilitation center, El Cedron (Tarso) at 5-6 miles from the center, and San Francisco (Pueblorrico) at 8 miles from the center. The participatory assessment consisted of seven phases: 1) Introduction of the appraisal team (principal investigator – Paula A. Castaño) to the community; 2) 1st transect walk through the community (observation); 3) recruitment process for individual questionnaires and focus group meetings; 4) answer of individual questionnaires; 5) focus group meetings; 6) 2nd transect walk through the community (observation), and 7) data analysis.

The complete data analysis results of the participatory methods conducted during the study are still pending. Currently we have the data organized into five matrices, which were identified as the major source of income/profession for the communities evaluated. The matrices correspond to the following: 1) housekeeping, 2) farmer, 3) livestock producer (dairy), 4) farmer-livestock producer, and 5) other. The latter category represent those whose profession or source of income cannot be categorized under any of the other matrices and it includes retired and animal caretakers. The data organized under the matrices is currently in the process of coding. We are applying a coding system consisted of 98 codes. The coding system was obtained from the project goals and the information collected through the data provided by the community during the questionnaires, focus group meetings, and observations. Once the coding is completed a more detailed analysis will be conducted and conclusions will be made in relation to the association or non-association of human activity, behavior, and degree of interaction with NHP to known yellow fever risk factors and possible infection with the virus in these communities.

Preliminary data results:
A total of 72 persons participated from the study including women and men, 40 of them participated in the focus group meetings and 32 in the individual questionnaires. It should be noted that some of the participants who participated in the focus group meetings also participated in the individual questionnaires. However, as the identity of the participants was not determined, quantifying the number of crossover participants between the two participatory methods is not possible. More details on the number and gender of the participants in the focus groups and individual questionnaires by community are shown in Table 1.

Table 1. Number and gender of the participants in focus groups and individual questionnaires by communities

		Focus groups*		**Individual questionnaire**		**Total**
Municipality	Community	Gender	Number of participants	Gender	Number of participants	Participants/ category
Jericó	La Cascada	Male	2	Male	3	5
		Female	4	Female	7	11
	Patudal	Male	6	Male	3	9
		Female	4	Female	4	8
Tarso	El Cedrón	Male	1	Male	3	4
		Female	11	Female	4	15

Pueblorrico	San Francisco	Male	7	Male	6	13
		Female	5	Female	2	7
Total male participants			16		15	31
Total female participants			24		17	41
Total participants			**40**		**32**	**72**

* The number and gender of focus group's participants is determined based on observations and field notes made during the meeting.

Project budget

The table below represents the approximate cost associated with this pilot research project conducted in 2012-2013. TIE funds were used to pay travel/lodging/food expenses in Brazil and Colombia, field project supplies, project permits, and sample analysis. It is also important to note that the principal investigator (Paula A. Castaño) had to pay 15% on taxes for the money granted before the funding was available, as a result of her non-resident alien status in United States.

Total Grant awarded: $ 5860

Expenditures TIE fellowship 2012-2013			
Category	**Description**	**TIE Budget (US$)**	**Project Cost (US$)**
Tax deduction – nonresident alien	Grant money less 15% tax	894	894
Travel	US - Brazil	690	690
	Brazil ground transportation	110	200
	Brazil - Colombia	596	596
	Colombia air and ground transportation	450	700
	Colombia - US	403	403
	Travel insurance	286	500.5
	Travel Total	**2535**	**3089.5**
Lodging/ Food	Brazil	210	400
	Colombia	560	950
	Lodging Total	**770**	**1350**
Supplies	Biological sampling	300	300
	Participatory assessment	200	200
	Supplies Total	**500**	**500**
Project permits - Colombia	Governmental authorities	91	91
	Newspaper publication	170	200
	Project permits Total	**261**	**291**
Sample analysis	Universidad de Antioquia (Hematology, fecal, and blood smears)	200	200

	National Institute of Health (Serum analysis - Yellow fever antibodies)	700	700
	Sample analysis total	**900**	**900**
Total expenditures		**5860**	**7024.5**

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