

THE ROLE OF PROTOZOAN GENOTYPIC DIVERSITY IN HUMANS: IMPLICATIONS FOR THE EPIDEMIOLOGY OF CRYPTOSPORIDIOSIS AND GIARDIASIS IN NEW ZEALAND

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VETERINARY,
ANIMAL AND
BIOMEDICAL
SCIENCES



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^mEpiLab



Massey University
Protozoa Research Unit

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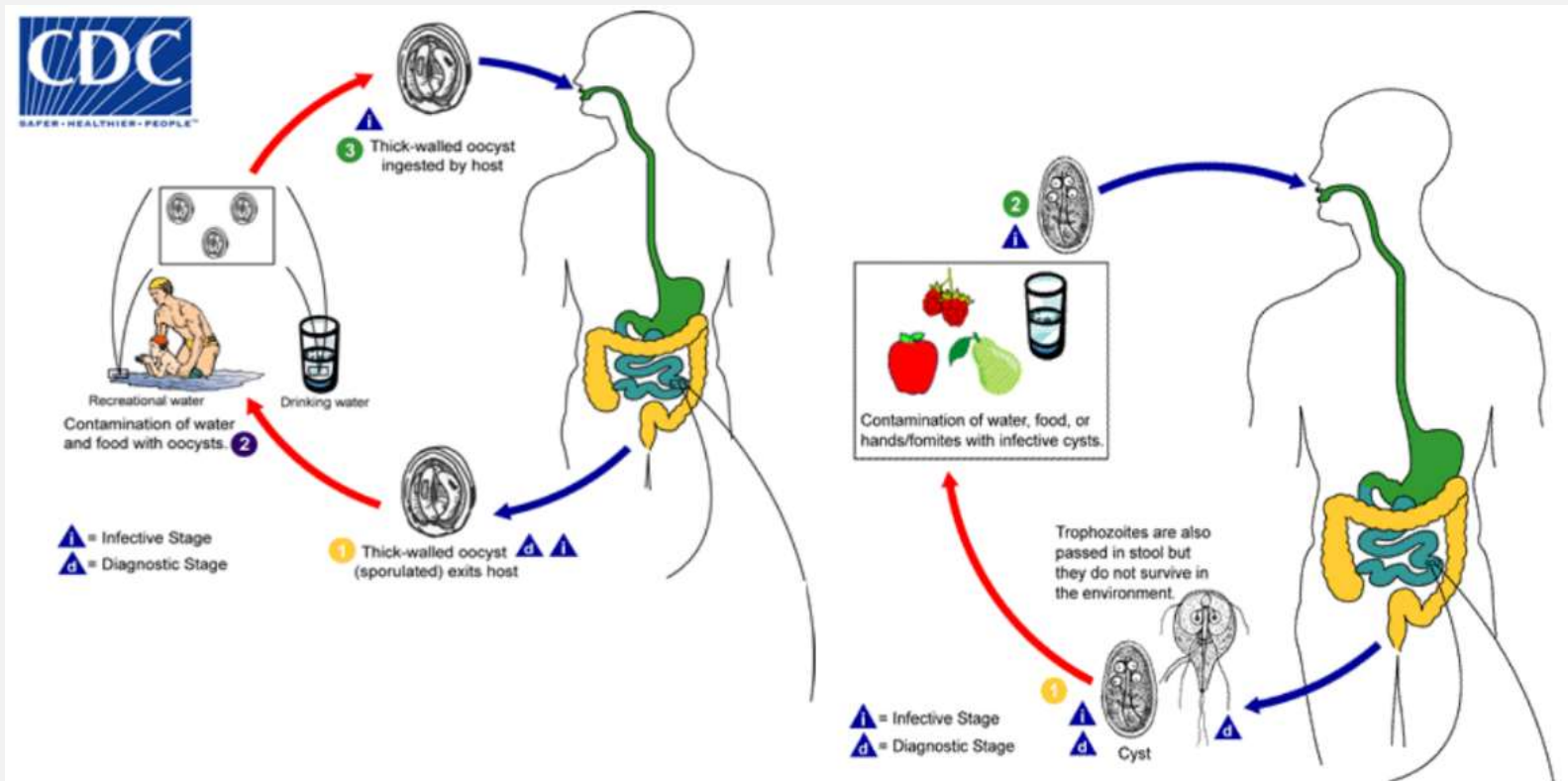
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THE DIARRRHEIC DUO

- *Cryptosporidium*:
 - Phylum *Apicomplexa*
 - 38 species in various vertebrate hosts
- *Giardia*:
 - Phylum *Metamonada*
 - 51 species in vertebrate hosts



IMPORTANCE

- Global impact
 - *Cryptosporidium* and *Giardia* can cause death in immunocompromised individuals like those infected with HIV
 - Diarrhoea was fourth leading cause of death among children younger than 5 years
 - No suitable vaccines against either parasite
- NZ impact
 - In 2016 *Cryptosporidium* and *Giardia* were responsible for 5.4% and 7.4% of outbreaks:

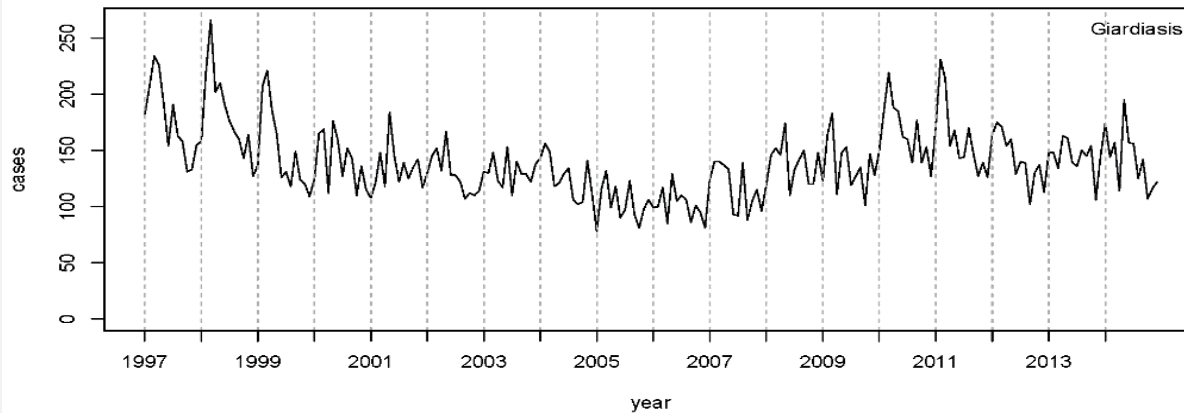
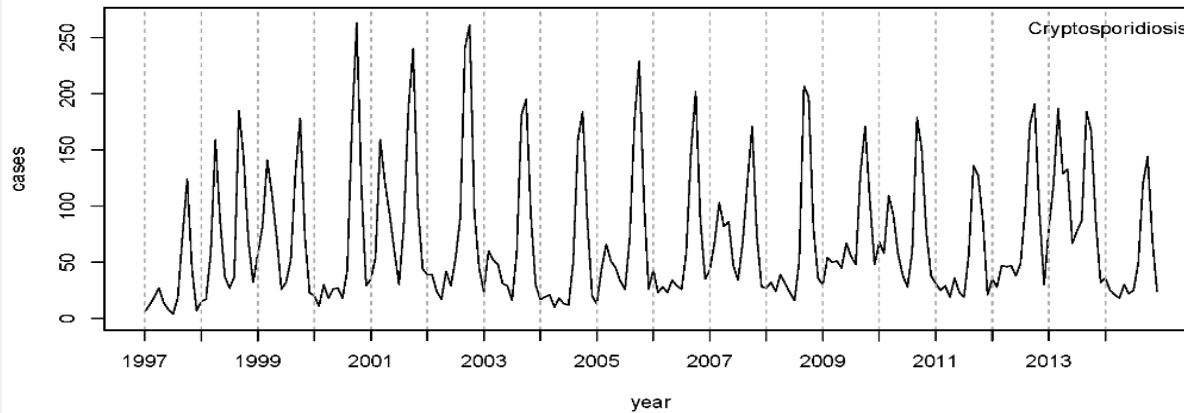
SEASONALITY

C. hominis:

- Humans

C. parvum:

- Humans
- Livestock
- Pets
- Wildlife
- Rodents

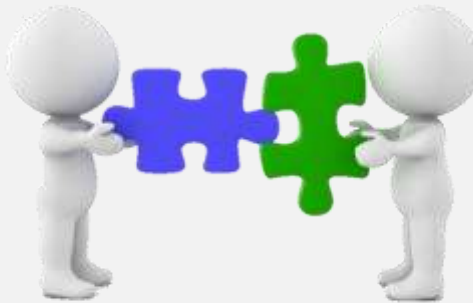


G. intestinalis:

- Humans
- Livestock
- Pets
- Wildlife

HOST-PATHOGEN INTERACTIONS

- Species and subtype specific differences in host range and human infectivity - growth rates & intestinal distribution
- Clinical effects of both diseases in humans are highly variable
- Public health risk usually estimated by oocysts densities not considering infectivity or genotype



- Question remains as to whether contact rates or protozoa genotype determine case numbers and disease outcome
- At the moment in NZ there is an inability to identify same subtypes of protozoa in epidemiologically linked cases.

HYPOTHESES

- H_1 : Epidemiologically linked cases share subtypes undetectable with consensus PCR and Sanger sequencing
- H_2 : Host-pathogen interactions are determined by genetic differences at the between species level
- H_3 : Host-pathogen interactions are determined by ecological differences at the between genotype level

H₁: COMPARISON OF NEW ZEALAND HUMAN PROTOZOAN OUTBREAKS USING AMPLICON-BASED METABARCODING TO IDENTIFY COMMON LINEAGES AND WITHIN HOST DIVERSITY

Aim:

- To use amplicon-based metabarcoding to resolve protozoa outbreak epidemiology

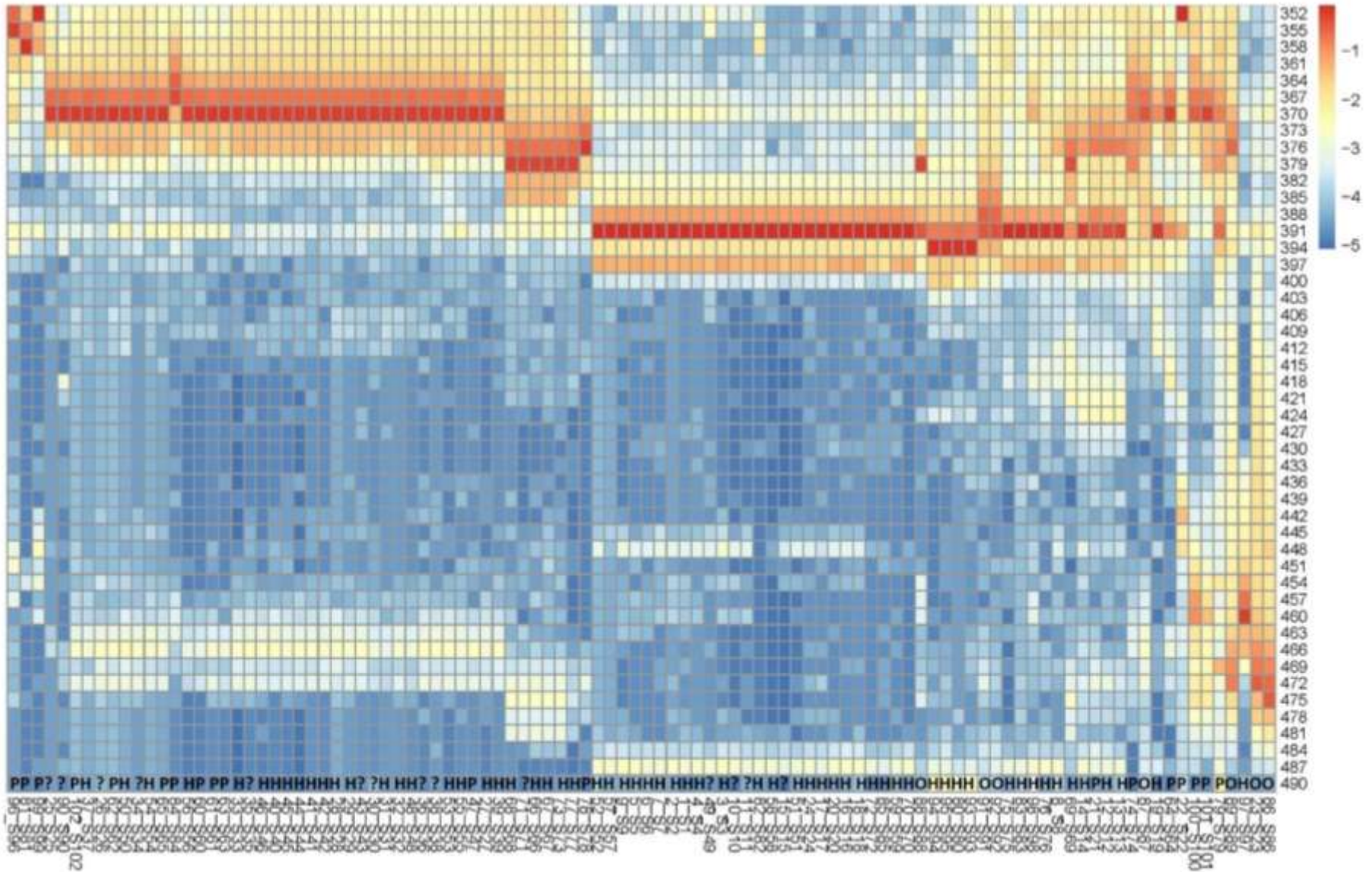
Materials and Methods:

- Outbreak samples from 2010 to 2018
- Samples from routine surveillance
- Previously verified by PCR and Sanger to be positive for both parasites
- Illumina MiSeq™ Sequencing
 - Loci: Glycoprotein 60 (*Cryptosporidium*), Glutamate dehydrogenase (*Giardia*)

C. parvum?

C. hominis?

Mixed?



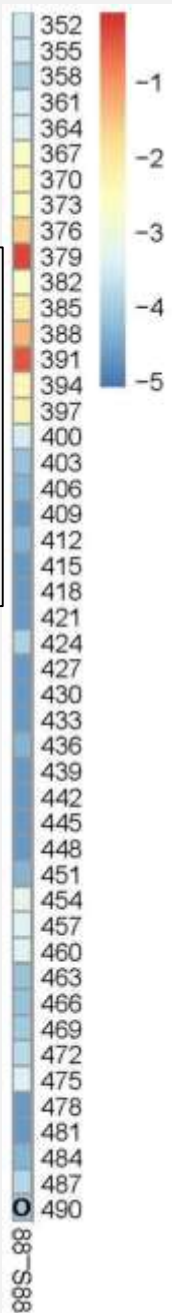
Sample 88

Sanger: IXb (*C. tyzzeri*)

NGS:

IXb (*C. tyzzeri*)

IbA10G2 (*C. hominis*)



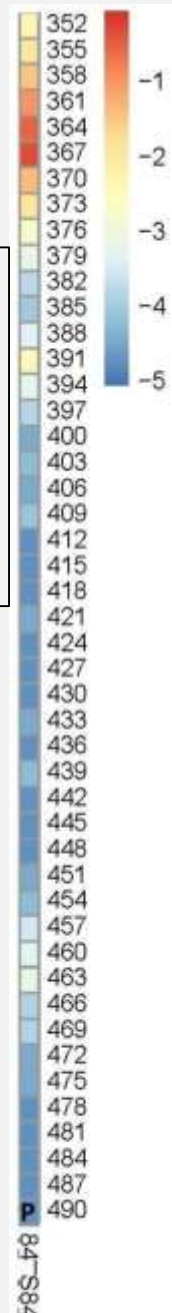
Sample 84

Sanger: IldA24G1 (*C. parvum*)

NGS:

IbA10G2 (*C. hominis*)

Ild (*C. parvum*)



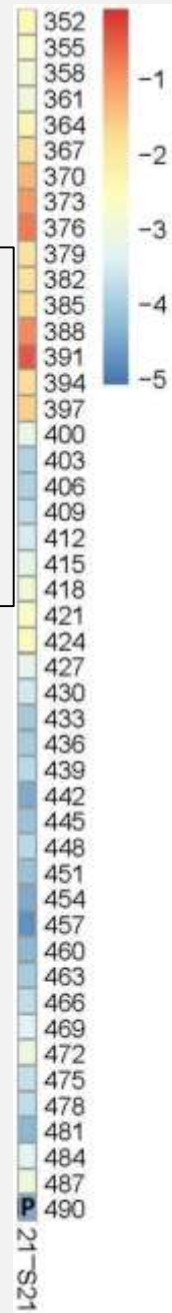
Sample 21

Sanger: IlaA18G3R1 (*C. parvum*)

NGS:

IbA10G2 (*C. hominis*)

IlaA18G3 (*C. parvum*)



FUTURE WORK

- Characterisation of sequences
- Error correction and cluster analysis
- Comparative analysis to Sanger sequence data

H_{2&3}: COMPARATIVE ANALYSIS OF FACTORS INFLUENCING *CRYPTOSPORIDIUM* AND *GIARDIA* INFECTIVITY

Aim:

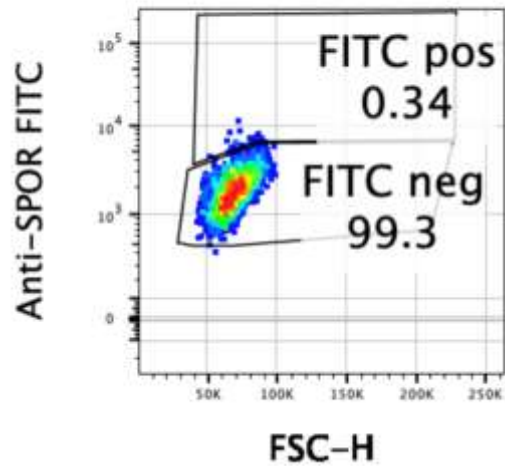
- To compare the infectivity and pathogenicity between isolates in vitro

Materials and methods:

- Human cell culture:
 - *Giardia*: Caco2
 - *Cryptosporidium*: COLO-680N
- Developing (oo)cysts purification methods
- Flow Cytometry:
 - Distinguish between infected and uninfected populations

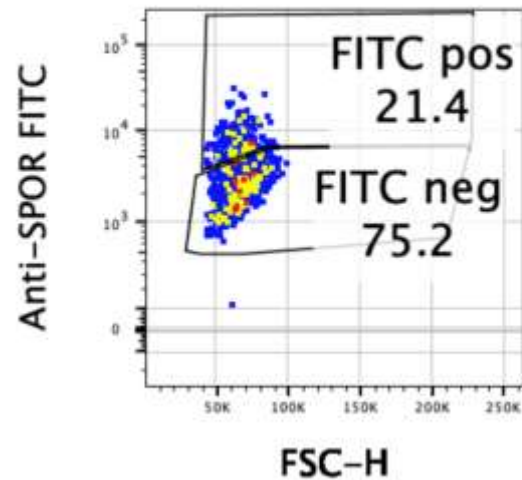
COLO-680N Anti-Spor FITC Conc E

Single Cells
4723



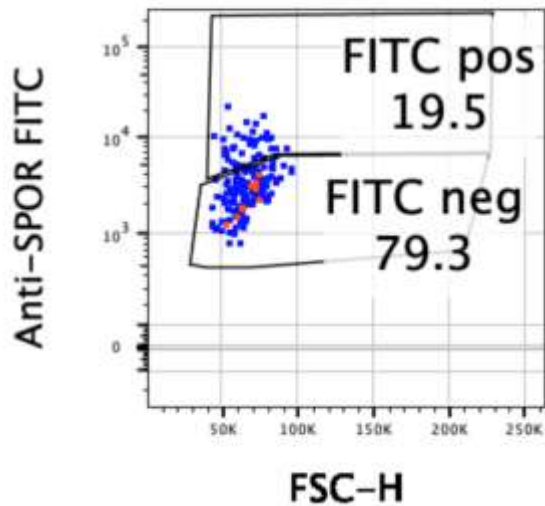
Parvum:COLO - 100:1 Rep 3

Single Cells
747



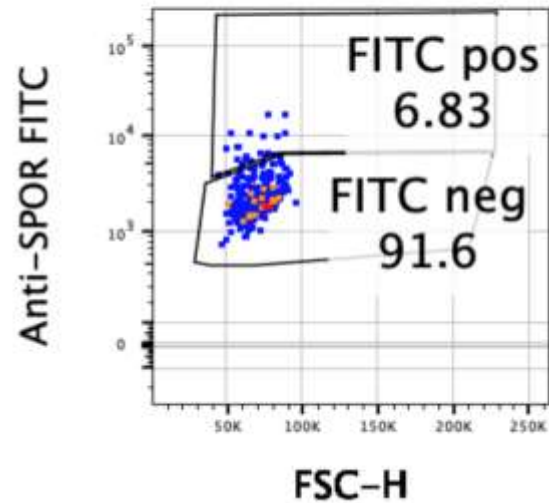
Parvum:COLO - 100:1 Rep 1

Single Cells
174



Hominis:COLO 100:1 Rep 3

Single Cells
249



FUTURE WORK

- Measure immune response from infected cells
- Measure the change in abundance of genotypes present over the course of *in vitro* infection
- Transcriptomic analysis to ascertain gene expression factors behind infectivity
- Whole genome sequencing to identify areas under positive selection

A scenic view of a pond with ducks and reeds, framed by autumn trees and a building in the background. The pond is filled with water, and several ducks are visible swimming. The foreground is dominated by tall, green reeds. In the background, there are large trees with yellow and orange autumn foliage, and a building with blue accents is partially visible. A white car is parked on the right side of the image.

THANK YOU