Seroprevalence of anti-*Kudoa* sp. (Myxosporea: Multivalvulida) antibodies in a Canary Island population (Macaronesia-Spain)

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Content

1. Introduction

1.1. Fisheries situation in the Canary Islands.

The Canary Islands are home to an ecosystem with a remarkable variety of marine resources, the result of the geographical, oceanographic and physical particularities of the region. However, this environment is extremely delicate. One of the elements that influence the configuration of this ecosystem is the steep slope of the seabed, giving rise to small island platforms. This restricts the habitable surface area for coastal species such as sama, vieja, cabrilla, grouper or abade; however, it favours the presence of pelagic species such as tuna, sardines and mackerel.(Gobierno de Canarias, Consejería de Agricultura, Ganadería y Pesca, 2023).

Most of the boats used for professional fishing in the Canary Islands are around 15 metres long. Each of these vessels uses specific fishing methods aimed at different marine species. There are also other vessels of larger dimensions that focus on catching species such as large tuna. Consequently, we can affirm that professional fishing in the Canary Islands is characterised by its artisanal nature, its versatility and its multi-species approach. (Gobierno de Canarias, Consejería de Agricultura, Ganadería y Pesca, 2023).

In the Canary Islands, the practice of aquaculture activities is also carried out, which consists of the controlled breeding or cultivation of aquatic species with the aim of increasing production beyond the natural capacities of the environment. The most predominant method of aquaculture in the Canary Islands is the fattening of fish in cages located in marine installations. The latest data from the Ministry of Agriculture, Livestock and Fisheries for 2022 indicate that there was a fresh fish production of 7,412,577.71 kg (Gobierno de Canarias, Consejería de Agricultura, Ganadería y Pesca, 2023).

1.2. Traceability

This traceability and labelling of seafood products are considered fundamental elements to safeguard the interests and health of consumers who play an important role as through their purchasing choices and preferences they can purchase fish from reliable and sustainable sources and have a significant impact on promoting the shift towards traceability and sustainability. Traceability is supported by a labelling system that provides transparency and helps to prevent illegal, unreported and unregulated fishing. On the other hand, the lack of adequate regulation and controls can lead to fraudulent practices with a series of negative consequences such as fish poisoning (Paolacci et al., 2021).

1.3. Adverse reactions parasites

In recent years, the consumption of raw fish has increased markedly in many countries, becoming a new culinary habit (Morozińska-Gogol, 2019) and the consumption of this raw fish poses considerable risks to consumers. Fish allergens, contaminants such as parasites capable of triggering allergic reactions have been identified. Exposure to the proteins of these parasites, either live or dead, can induce allergic responses in certain individuals (Sharp and Lopata, 2014). This phenomenon underlines the importance of considering both intrinsic fish allergens and external elements, such as parasites, when assessing food safety and preventing adverse reactions in consumers (Morozińska-Gogol 2019).

There are a variety of parasite groups that can infect fish and some of these have species that have been shown to be zoonotic (Williams et al., 2020). The possibility of unintentional ingestion of zoonotic parasites has been noted, although comprehensive research has not yet been carried out to consider these parasites in fish as a human health concern (Williams et al., 2020). Different species of fish, spanning a wide taxonomic diversity, can transmit these parasites with varying life cycles and transmission patterns.

1.4. *Kudoa* spp.

Among the parasites present in commonly consumed fish are the Myxosporidia. The class Myxosporea belong to the phylum Cnidaria. These parasites mainly affect fish, but can also occur in reptiles, amphibians and higher animals. Myxozoa can be hosted by both fish (vertebrates) and annelids (invertebrates). They have a complex life cycle (Correya et al. 2021).

Within the family *Kudoidae* which includes myxosporean parasites, the described species belonging to the genus *Kudoa* and can infect a wide variety of marine teleosts. This has direct consequences for fisheries and aquaculture as the presence of these parasite species can cause pathological damage to the musculature of the fish, reducing the market value of these products because visually they can produce a rejection by consumers (Rodríguez-Ponce et al., 2019).

Kudoa species are capable of producing spore pseudocysts in the somatic muscle of marine and estuarine fish (Martínez De Velasco et al. 2007). A "pseudocyst" is a structure that, according to the definition of Lom and Dyková (1995), resembles a cyst or a lesion that bears a remarkable resemblance to a cavity surrounded by a dense fibrous capsule. Unlike conventional cysts, the pseudocyst does not have an inner lining formed by the parasite. Instead, it is characterized by a continuation in formation with necrotic changes, where the wall develops due to reparative inflammation. Anderson (1985) also used the term pseudocyst to describe species that reside in the host muscle tissue without a cellular envelope (Moran et al. 1999).

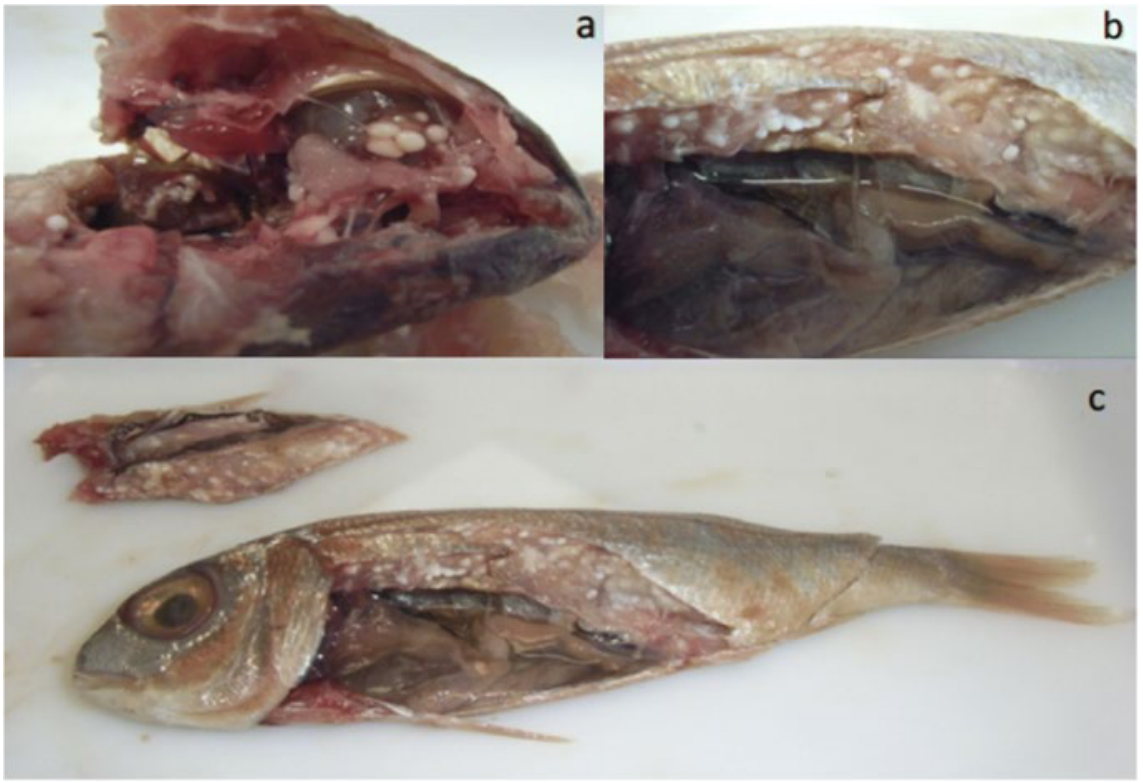


Fig.1. *Photos of mature Kudoa cysts in situ in skeletal muscle of Pagellus acarne* (Rodríguez-Ponce et al., 2019).

Some of the parasite species described in this genus have been identified as pathogenic for humans as they pose a risk to our health because of the possibility of food poisoning and/or allergic reactions (Rodríguez-Ponce et al. 2019).

1.5. "*Kudoa* food poisoning".

Food poisoning attributed to the ingestion of any species belonging to the genus *Kudoa* is particularly associated with the consumption of fresh raw fish, such as the popular Japanese dish sashimi (Sugita-konishi et al., 2014). The condition has raised concerns in Japan as it manifests with symptoms such as diarrhoea and vomiting within 12 hours of eating the contaminated food. Fortunately, symptoms tend to subside within 24 hours, giving the disease a generally favourable prognosis with no lingering sequelae (Sugita-konishi et al., 2014). In 2011, the Japanese Ministry of Health, Labour and Welfare issued a notification identifying the causative agent of this foodborne illness as

a previously unknown parasite called *Kudoa septempunctata* (Myxozoa: Myxosporea: Multivalvulidae). This finding has contributed to a better understanding of the potential public health implications and control measures for this disease (Sugita-konishi et al. 2014).

1.6. Host-parasite interactions

Some species of the genus *Kudoa* are capable of producing proteolytic enzymes that destroy muscle fibre. When inside a muscle fibre, the host immune system, in this case the fish, does not detect it. The parasite continues to grow until it breaks the sarcolemma and is recognised by the fish (Martínez De Velasco et al. 2007). The main defence mechanism of fish against the parasite is inflammation. But it is not observed until it reaches the sporogonia and the plasmodium containing mature myxospores. Once inflammation has occurred, the myxospores are ingested by macrophages and then destroyed (Moran et al. 1999).

These cysts or 'pseudocysts' are of great economic importance in the production and sale of fish. In fact, some species of *Kudoa*, such as *K. thyrsites*, have the ability to form pseudocysts macroscopically observable in the muscle of the host fish, resulting in a syndrome known as 'soft flesh', 'milky flesh' or 'jelly flesh'. This situation leads to a postmortem myoliquefactive degeneration that negatively affects the consumer's perception of the texture of meat from infected species (Iglesias et al. 2022).

The genus *Kudoa* is composed of approximately 100 named species (Shin et al., 2016). In Spain, *Kudoa*-infected specimens in several commercial fishes species have been detected and some of them cause myoliquefaction. However, this process has not been detected in the fish species analysed in the Canary coasts affected by *Kudoa*, despite the fact that many species of fish present pseudocysts in the musculature, a presence which, in itself, is already unfavourable for the consumer.

It was also previously shown that *Kudoa* spp. pseudocysts developed high levels of IgG1 and IgE antibodies in BALB/c mice, suggesting that some of the components of this parasite may be allergenic and could therefore be a risk to human health (Martínez De Velasco et al., 2007).

1.7. Taxonomy

The family Kudoidae belongs to the order Multivalvulida within the class Myxosporea. The order Multivalvulida is described as containing species having myxospores composed of more than two valves (Moran et al. 1999).

The genus of the family Kudoidae with myxospores with more than 4 leaflets and polar capsules is *Kudoa* (Myxosporea: Multivalvulida)(Moran et al., 1999), where most of its more than 100 nominal species (Shin et al., 2016), are histozoan myxozoans, characterised by square or stellate myxospores with delicate membranes and indistinct sutures between the four leaflets (Figure 2) that affect several fish species by infecting their musculature (Moran et al. 1999).

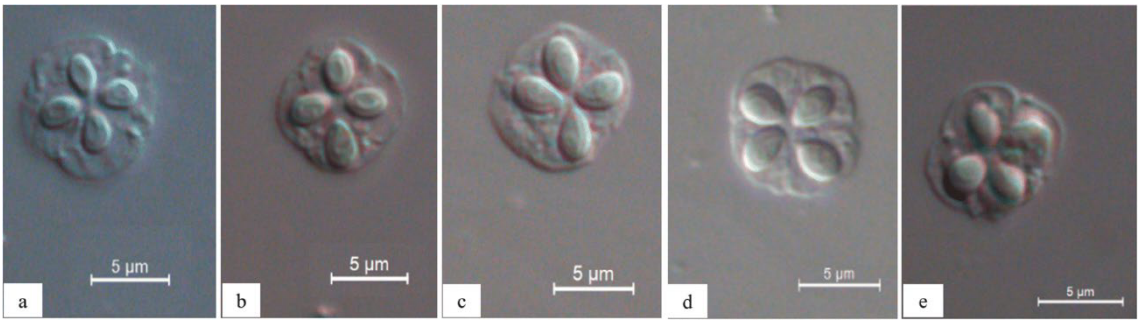


Fig. 2. DIC photomicrographs of fresh spores in apical view of *Kudoa* sp. *from Serranus cabrilla (a), Sarpa salpa (b), Spondyliosoma cantharus (c), Pagellus acarne (d),* and *Pagellus erythrinus (*e) (Rodríguez-Ponce et al., 2019).

1.8. Morphology

Initially the characterisation of *Kudoa* parasites was based on spore morphology and morphometry. The problem was that these morphological features were limited and characterisation cannot be completely dependent on the shape and dimensions of the mature spore. Finally, this classification has been revised by considering ribosomal DNA (rDNA) information, resulting in a much more accurate identification (Rodríguez-Ponce et al. 2019).

There is currently no effective method to detect fish parasitised with *Kudoa* without destroying them, so it is inevitable that infected fish will reach consumers. Although these pseudocysts are white or black in colour in the fish flesh, they often go undetected (Martínez De Velasco et al. 2007).

1.9. *Kudoa* spp. life cycle

In the genus *Kudoa*, myxospore development occurs in the host fish .(Moran et al., 1999), within its muscle fibres, as a plasmodium, a multinucleate stage of parasite growth, and appears to undergo no cell divisions. Instead, it grows considerably to a remarkable size, filling with numerous developing myxospores. Specifically in the genus *Kudoa*, sporogenesis, has been the subject of detailed investigations using transmission electron microscopy techniques. These studies have focused on species such as *K. lunata* and *K. paniformis*, providing crucial information on the biology and life cycle of these parasites (Moran et al., 1999).

1.10. Objectives

After the detection of certain batches of fish with high parasite loads and considering the possibility of analysing sera from people from all over the archipelago, we decided to study the seroprevalence against IgG and IgE for this group of parasites.

For this purpose, this study investigated the seroprevalence of antibodies against *Kudoa* spp. in order to know the immune status of a population sample from the seven Canary Islands. IgG and IgE levels were determined by ELISA.

The antigen used was derived from cysts extracted from five species, in which the genus *Kudoa* has been identified, from the Canary coasts: *Pagellus acarne, Pagellus erythrinus, Serranus cabrilla, Sarpa salpa* and *Spondyliosoma cantharus*.

2. Material and Methods

2.1. Subjects, blood samples

The study was carried out using a random selection of sera from the population of the seven Canary Islands which were collected between March 2014 and October 2015. These sera had been previously used in a previous investigation (Cabrera et al., 2018), and were obtained from several diagnostic laboratories. All participants gave their consent to take part in this study and confidentiality of patient information was always maintained.

For preservation, sera were stored at -80°C until analysis. The data collected from each of the serum samples used included information such as age, sex and place of residence of the participants. The distribution of samples by age and sex reflected the population structure of the Canary Islands, based on 2015 data (ISTAC, 2015).

2.2. *Kudoa* spp. Antigen and determination of specific antibodies

Kudoa antigen is derived from pseudocysts obtained from specimens of *Pagellus acarne, Pagellus erythrinus, Serranus cabrilla, Sarpa salpa* and *Spondyliosoma cantharus*. These pseudocysts were subjected to a homogenisation process by sonication for 6 minutes (at a frequency of 10 s/pulse), followed by extraction in PBS at a temperature of 4°C overnight. Subsequently, delipidisation with n-hexane and centrifugation at 8497 g for 30 min at 4°C (Biofuge 17RS, Heraeus Sepatech) was performed. The resulting supernatant was dialysed overnight at 4°C in PBS. and subsequently titrated by Bradford's method to determine its protein concentration.

2.3. ELISA

2.3.1. IgG

ELISA plates (Costar, Corning, NY, USA) were used to assess specific antibody levels. The first four rows of the plate were coated with a 10 µg/mL concentration of *Kudoa* antigen, while the remaining rows were left uncoated to serve as a control for non-specific reactions. Subsequently, the plate was incubated overnight at a temperature of 4°C.

Plates were washed three times with 0.05% PBS-Tween 20 (PBS-Tween) after 24 hours and blocking was performed by adding 200 µl per well of 0.1% BSA (Sigma, St Louis, MO, USA) in PBS, incubating for 1 h at 37°C.

After washing by the same method as above, 100 µl of serum samples diluted 1/100 in PBS-Tween solution with 0.1% BSA were added in duplicate. These samples were incubated at 37°C for a period of 2 hours.

After the plate washing process was completed, for IgG, 100 µl of an affinity-isolated anti-IgG antibody, which was labelled with goat peroxidase (IgG Fc Goat anti-Human, HRP, Invitrogen), was added at a dilution of 1/8000 in PBS-Tween solution with 0.1% BSA. These plates were incubated for 1 hour at a temperature of 37°C.

A final wash gave way to the addition of 100 µl per well of a 0.04% substrate (Ophenylene-Specific diamine; Sigma, St Louis, MO, USA) in 0.04% phosphate-citrate buffer at pH 5.0 together with 0.04% hydrogen peroxide. The reaction was then stopped by the addition of 50 µl of normal H2SO4 and the plates were read at a wavelength of 490 nm using a HEALES spectrophotometer model MB-580.

2.3.2. IgE

ELISA plates (Costar, Corning, NY, USA) were used to assess specific antibody levels. The first four rows of the plate were coated with a 10 µg/mL concentration of *Kudoa* antigen, while the remaining rows were left uncoated to serve as a control for non-specific reactions. Subsequently, the plate was incubated overnight at a temperature of 4°C.

Plates were washed three times with 0.05% PBS-Tween 20 (PBS-Tween) after 24 hours and blocking was performed by adding 200 µl per well of 0.1% BSA (Sigma, St Louis, MO, USA) in PBS, incubating for 1 h at 37°C.

After washing by the same method as above, 100 µl of serum samples diluted 1/2 in the case of IgE measurements were added in PBS-Tween solution with 0.1% BSA in duplicate. These samples were incubated at 37°C for a period of 2 hours.

After washing of the plates, 100 µl per well of an unlabelled monoclonal anti-human IgE antibody (IgG1: Human IgE-Anti immunoglobulin E, specificity against human IgE Fc-isotype IgG1, Eurofins. Ref M.30.000.IE21A11 1ml) in a dilution of 1/1000 of PBS-Tween with 0.1% BSA. We then washed, and developed with a peroxidase-labelled murine goat anti-mouse IgG1 antibody (Goat anti mouse IgG1 (Y1), Horseradich peroxidase conjugate Ref A10551 from Invitrogen by Thermo Fisher Scientific, 0.5 mg), also in a 1/1000 PBS-Tween solution with 0.1% BSA. Both conjugates were incubated for 1 hour at 37°C each.

A final wash gave way to the addition of 100 µl per well of a 0.04% substrate (Ophenylene-Specific diamine; Sigma, St Louis, MO, USA) in 0.04% phosphate-citrate buffer at pH 5.0 together with 0.04% hydrogen peroxide. The reaction was then stopped

by the addition of 50 µl of normal H2SO4 and the plates were read at a wavelength of 490 nm using a HEALES spectrophotometer model MB-580.

2.4. Samples Classification

Data obtained from each serum sample included information on age, gender and place of residence. The samples were classified into five different age groups, each covering a 15-year interval. The last group corresponded to people aged 60 years and older, including the oldest person in the study.

2.5. Climate Classification

A classification of the samples according to the different climatic zones of the archipelago was carried out using the Köppen classification, also known as the Köppen Geiger classification, as described by (Rodriguez-Ponce et al., 1995).

The Köppen-Geiger classification is used to describe the different climate types based on average monthly precipitation and temperatures. This classification establishes specific temperature and precipitation ranges that have an important impact on the distribution of vegetation and human activity.

The following climate types have been identified in the Canary archipelago using this classification:

2.5.1. Dry zones

Dry zones are found in the regions below 200 metres or near the coast of the Canary archipelago. These areas are divided into two subclimates: the dry desert (Dd) and the dry steppe (Ds). These dry varieties are more common on the islands of Fuerteventura and Lanzarote.

The dry desert subclimate (Dd) is characterised by precipitation below the annual average for the archipelago, an average annual temperature above 18ºC and a very dry summer. On the other hand, the dry steppe subclimate (Ds) has the same characteristics, but with a more steppe-like climate.

2.5.2. The temperate zones

This type of climate is known as mesothermal climate, and is characterised by having a higher amount of rainfall, mainly during the coldest months, and an average temperature below 18ºC during the winter. These zones are subdivided into two subclimates: mild temperate (Tm) and cold temperate (Tc).

The Tc subclimate is characterised by hot, dry summers and mild winters. It is widespread at altitudes between 200 and 800 metres. On the other hand, the Tm subclimate is found at higher elevations, generally above 800 metres. It is similar to the Tc subclimate but with the difference that summer temperatures are usually below 22ºC and winters are colder (*Atlas climático de los archipiélagos de Canarias, Madeira y Azores*, 2012).

These climatic varieties are most frequently found in the islands of El Hierro, La Gomera and La Palma.

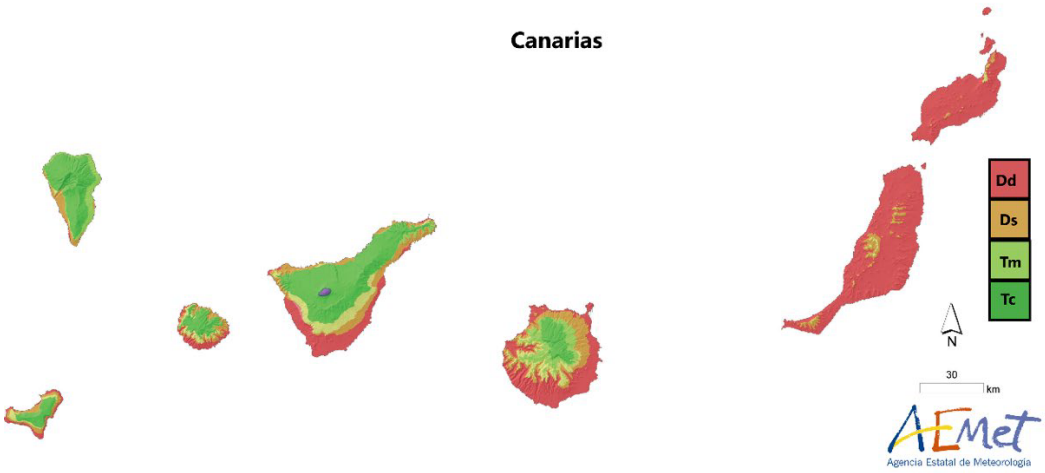


Figure 3. *Köppen-Geiger climate classification in the Canary Islands* (Climate atlas of the archipelagos of the Canary Islands, Madeira and the Azores, 2012).

2.6. Statistical analysis

For the analysis of each sample, four results were obtained by ELISA (two in the antigen-sensitised wells and two in the wells not sensitised with the antigen). The final result (FR) was calculated using the following formula:

$$FR = \frac{(A1 + B1) - (E1 + F1)}{2}$$

Where wells A1 and B1 (green) were sensitised with the *Kudoa* antigen and wells E1 and F1 (red) were kept unsensitised, containing only BSA.

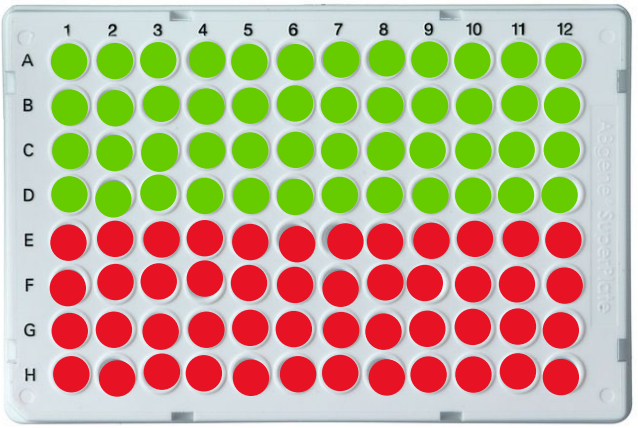


Fig.4. *ELISA plate. Sensitised wells (green), unsensitised wells (red).*

Results that equalled or exceeded the sum of the mean plus twice the standard deviation of all samples were considered positive.

The analysis of the data obtained was made using RStudio® (version 2022.02.1 Build 461) and Excel® Microsoft Office 365®.

3. Results

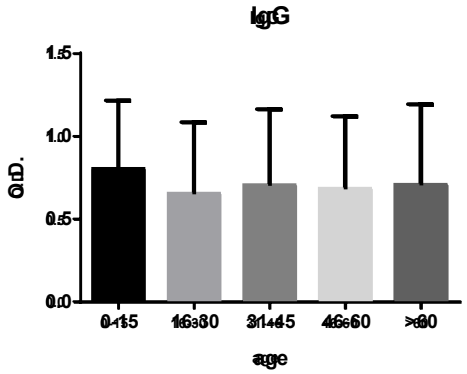
Out of 1151 sera studied, 108 were eliminated due to incomplete data, so the final sample size was 1043. The samples by islands can be seen in the Table 1, where the seropositive samples by sex are also listed. The last lines represent the total number of positives for each immunoglobulin and the corresponding percentage.

	N	IgG POSITIVE		IgE POSITIVE	
		WOMEN	MEN	WOMEN	MEN
Gran Canaria	94	0	2	0	0
Tenerife	217	9	3	2	1
Fuerteventura	214	7	9	0	1
Lanzarote	187	3	2	5	2
La Palma	187	13	16	3	2
La Gomera	64	0	2	1	0
El Hierro	80	0	3	0	10
Total	1043	32	37	11	16
Total positive Ig		**69**		**27**	
% Positive		**6.6**		**2.6**	

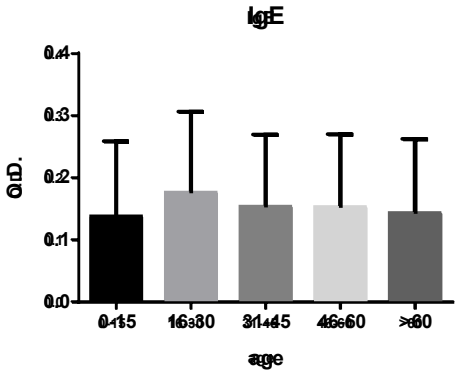
Table 1. Seropositivity by island, sex and immunoglobulins, where N is the sample of the population.

In view of the high percentage of seroprevalence found above all for IgG (6.6%) and somewhat lower for IgE (2.2%), it can be observed that they have the same relationship for both immunoglobulins with respect to sex, i.e. there are no differences between sexes for any of the immunoglobulins studied, highlighting the seropositivity for immunoglobulin G compared to IgE.

Looking at the results obtained for the optical densities with respect to the different age ranges, we obtain graphs 1 and 2.



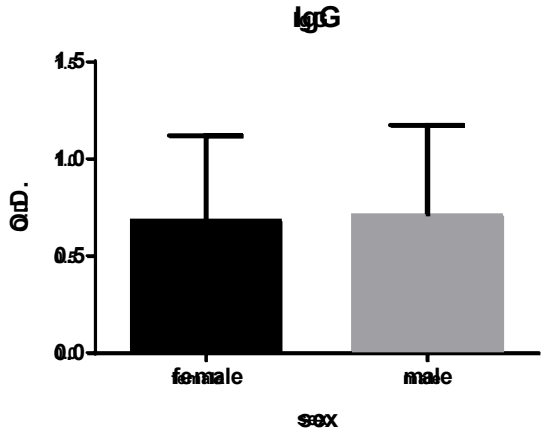
Graph 1. Optical density for IgG for different age ranges ($p<0.05$).



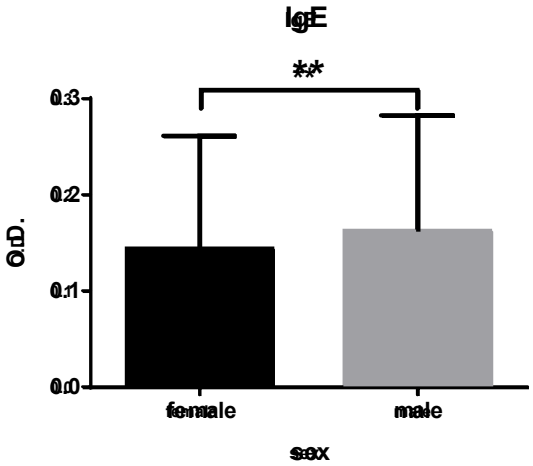
Graph 2. Optical density for IgE for different age ranges ($p<0.05$).

In both graphs, 1 for IgG and 2 for IgE, no significant statistical differences were observed, although it is noteworthy that the optical densities in IgG are higher than in IgE, as in table 1.

On the other hand, if we compare the optical density values with respect to sex, we obtain the following graphs (3 and 4).



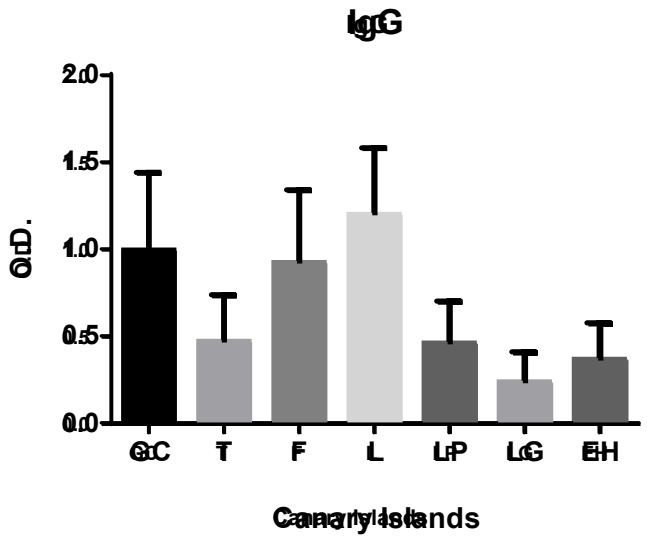
Graph 3. Optical density for IgG with respect to sex.



*Graph 4. Optical density for IgE with respect to sex **($p<0.01$).*

There are no significant sex differences for IgG (Graph 3), but there are significant sex differences for IgE (Graph 4) with a ($p<0.01$). IgG stands out with higher optical density values than IgE.

Respect to seropositivity on each island (Graphs 5 and 6), different levels of significance are shown in the side tables (table 2 and 3).



Graph 5. Optical density for IgG with respect to the different islands.

GC vs. T	****
GC vs. LP	****
GC vs. LG	****
GC vs. EH	****
T vs. F	****
T vs. L	****
T vs. LG	****
F vs. L	****
F vs. LP	****
F vs. LG	****
F vs. EH	****
L vs. LP	****
L vs. LG	****
L vs. EH	****
LP vs. LG	****

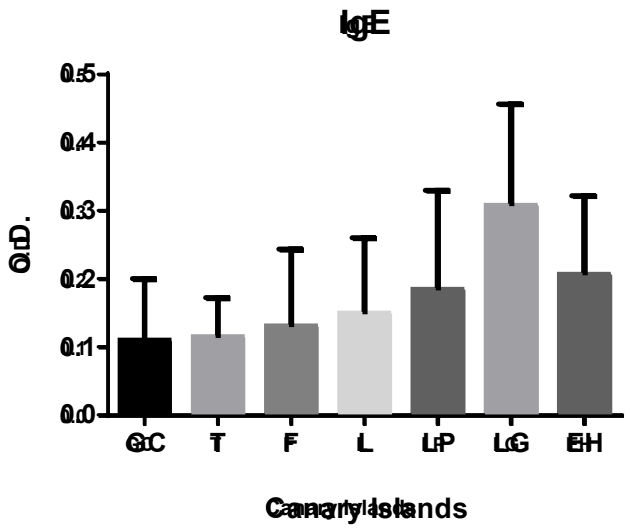
*Table 2. Significance levels for IgG. ****(p<0.0001).*

The results related to immunoglobulin IgG show significant statistical differences between various island pairs:

- Significance Level (****) (p<0.0001): highly significant statistical differences were found. These differences were found in the following island pairs:

  - Gran Canaria with Tenerife, La Palma, La Gomera and El Hierro.
  - Tenerife with Fuerteventura, Lanzarote and La Gomera.
  - Fuerteventura with Lanzarote, La Palma, La Gomera and El Hierro.
  - Lanzarote with La Palma, La Gomera and El Hierro.
  - La Palma with La Gomera.

The maximum optical density value obtained for IgG corresponds to the island of Lanzarote and the minimum value to La Gomera.



Graph 6. Optical density for IgE with respect to the different islands.

GC vs. L	*
GC vs. LP	****
GC vs. LG	****
GC vs. EH	****
T vs. LP	***
T vs. LG	****
T vs. EH	****
F vs. LP	***
F vs. LG	****
F vs. EH	****
L vs. LG	****
L vs. EH	***
LP vs. LG	****
LG vs. EH	*

*Table 3. Significance levels for IgE. *(p<0.05), ***(p<0.001), ****(p<0.0001).*

Table 3 shows significant differences in IgE immunoglobulin seroprevalence between different island pairs in the archipelago:

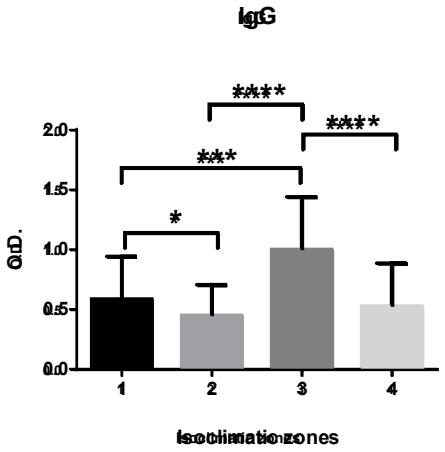
- Significance level: (*) (p<0.05): significant differences were observed between the islands of Gran Canaria and Lanzarote, as well as between La Gomera and El Hierro.

- Significance level (***) (p<0.001): significant differences were identified between the islands Tenerife and La Palma, as well as between Fuerteventura and La Palma, Lanzarote and El Hierro.

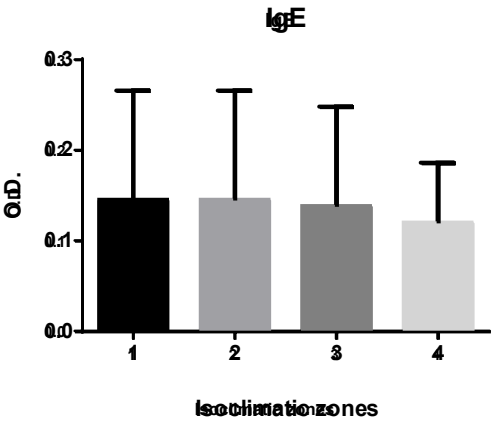
- Significance level (****) (p<0.0001): highly significant differences were found. significant differences were found. These differences involved several islands:

- Gran Canaria with La Palma, La Gomera and El Hierro.
- Tenerife with La Gomera and El Hierro.
- Fuerteventura with La Gomera and El Hierro.
- Lanzarote with La Gomera.
- La Palma with La Gomera.

Finally, the prevalence in the different isoclimates studied is shown in graphs 7 and 8 for immunoglobulins G and E respectively. Significant differences are observed for IgG (Graph 7) and none for IgE (Graph 8).



Graph 7. Optical density, mean and standard deviation for IgG respect to isoclimates. Zone 1: mild temperate, zone 2: cool temperate, zone 3: dry desert and zone 4: dry steppe.
(p<0.05), *(p<0.001), ****(p<0.0001).*



Graph 8. Optical density, mean and standard deviation *for IgE respect to isoclimates. Zone 1: mild temperate, zone 2: cool temperate, zone 3: dry desert and zone 4: dry steppe.*

For IgG (graph 7), it is classified according to its level of significance:

- ($p<0.05$): zone 1 (mild temperate) with zone 2 (cool temperate),
- ($p<0.001$): zone 1 (mild temperate) with zone 3 (dry desert).
- ($p<0.0001$): Zone 2 (cool temperate) with zone 3 (dry desert) and zone 3 (dry desert) with zone 4 (dry steppe).

4. Discussion

The results obtained in this seroprevalence study in the Canary Islands against the *Kudoa* parasite provide important information on the immunological response of the population to this pathogen. The distribution by islands, gender differences, the relationship between IgE and IgG immunoglobulins, the analysis by age groups and the influence of isoclimates on seroprevalence have been examined. Overall, a higher seropositivity for IgG (6.6%) compared to IgE (2.2%) was observed, suggesting a more persistent or past immune response to the parasite. (Schroeder and Cavacini, 2010) This difference could be related to the specific functions of each immunoglobulin, where IgG is associated with long-term responses, whereas IgE indicates an active or recent immune response, as well as possibly allergic sensitivity (Schroeder and Cavacini, 2010).

According to research carried out in Spain in 2019, there has been a 30% decrease in fish and seafood consumption since 2000, especially in the younger population. In earlier times, the average intake of these foods was around 88.9 grams per day per person, whereas today the average consumption is around 62.0 grams per day. Despite this decrease, Spain is still the country with the second highest consumption of fish and seafood in the world (Partearroyo et al., 2019).

In our study we have focused on the population of the Canary archipelago, in which in the year 2022, covering a period of 10 months, a fresh fish production of 7,412,577.71 kilograms was recorded, according to information provided by (Gobierno de Canarias, Consejería de Agricultura, Ganadería y Pesca, 2023).

In 2007, a study on the seroprevalence of anti-*Kudoa* sp. antibodies was carried out in Spain by (Martínez De Velasco et al., 2007) in a randomised Spanish population covering the regions of Asturias, Murcia and Seville. This study revealed a high prevalence of anti-

Kudoa antibodies in an apparently healthy population sample, with IgG, IgM, IgA and IgE antibody prevalences of 4.8%, 5.6%, 4.4% and 7.6%, respectively. In an additional study conducted in a hospital in Valencia by (Andreu-Ballester et al., 2008) , two groups were analysed: one composed of 80 people who underwent appendectomy and another control group of 80 people who only attended the emergency department. The seroprevalences of anti-*Kudoa* antibodies in the control group were 3.8% and 6.3% for IgG and IgE, respectively. A significantly lower seroprevalence for IgG was observed compared to the study by (Martinez de Velasco) and our analysis in the Canary Islands, which showed slightly higher figures, reaching 6.6%.

Despite the differences, both studies highlight the presence of anti-*Kudoa* antibodies in apparently healthy populations, indicating possible geographical variations in exposure and immune response to the parasite. In the case of IgE, higher figures were recorded in both studies, with 7.6% for the study by Martínez De Velasco et al. (2007) and 6.3% for the study by Andreu-Ballester et al. (2008) , while our analysis in the Canary Islands showed 2.2%. These discrepancies highlight the need for continuous monitoring and preventive measures to address public health in the context of fish production and consumption.

This study showed that there are no significant differences in terms of sex, coinciding with the study by (Andreu-Ballester et al., 2008) , in which no significant statistical differences in density values were observed between different age ranges (0-15, 16-30, 31-45, 46-60 and >60), in agreement with the aforementioned study which found no significant differences between age classes (20-30, 31-40, 41-50 and 61-76 years).

The analysis by islands reveals significant variations in IgG seroprevalence, with notable differences between several islands. The island of Lanzarote stands out with the highest optical density for IgG, while La Gomera stands out for IgE.

One might think that these results are supported by the intake of fish products, a study on Hg intake in the Canary Islands archipelago (Rubio et al., 2008) has been found in which Lanzarote ranks number 2 out of the 7 islands studied in the consumption of fish products; in contrast, La Gomera ranks number 6.

Differences in seroprevalence between islands may be related to various factors, such as geography, environmental exposure and genetic interactions. Comparison between isoclimates also reveals important information, highlighting significant differences in IgG seroprevalence between the mild temperate and cold temperate zones, as well as between the mild temperate zone and the dry desert. These results suggest that coastal or inland living and thus climatic conditions may be related to eating habits influencing the likelihood of acquiring more fish with the parasite in question.

In summary, this seroprevalence study in the Canary Islands provides detailed insight into the population's immune response to the *Kudoa* parasite. The findings highlight the importance of considering factors such as gender, geographical location and isoclimates when analysing seroprevalence. Furthermore, the results could have implications for the design of public health strategies and the implementation of specific preventive measures in regions with higher seropositivity.

5. Conclusions

- This study is the first investigation of the seroprevalence of anti-Kudoa antibodies in the human population of the Canary Islands, providing valuable insight into the immune response to this parasite.

- The overall seroprevalence of IgG against *Kudoa* was 6.6% and 2.2% for IgE, with some variation between islands. The island of Lanzarote stood out for IgG, while La Gomera showed the highest prevalence for IgE.

- Significant variations in seroprevalence between islands and different isoclimates highlight the possible influence of factors derived from fish consumption habits according to the area studied on the population's immune response to the *Kudoa* parasite.

- In order to better understand the seroprevalence and behaviour of the parasite in humans, further studies should be conducted with a larger population and differentiating between healthy and diseased individuals, as well as collecting more data such as the amount of fish products consumed in daily life.

References

Andreu-Ballester, J.C., Pérez-Griera, J., Ballester, F., Colomer-Rubio, E., Ortiz-Tarín, I., Pelayo, V., Rodero, M., Cuéllar, C., 2008. Anisakis simplex and Kudoa sp.: Evaluation of specific antibodies in appendectomized patients. Exp Parasitol 119, 433–436. https://doi.org/10.1016/j.exppara.2008.03.014

Atlas climático de los archipiélagos de Canarias, Madeira y Azores, 2012. . Agencia Estatal de Meteorología. https://doi.org/10.31978/281-12-006-X

Cabrera, E.D., Carretón, E., Morchón, R., Falcón-Cordón, Y., Falcón-Cordón, S., Simón, F., Montoya-Alonso, J.A., 2018. The Canary Islands as a model of risk of pulmonary dirofilariasis in a hyperendemic area. Parasitol Res 117, 933–936. https://doi.org/10.1007/s00436-018-5774-1

Correya, M.S., Vijayagopal, P., Sanil, N.K., 2021. Morphological and molecular description of a new species of Myxobolus (Myxosporea: Myxobolidae) infecting Planiliza macrolepis (Smith, 1846) from India. Journal of Parasitic Diseases 45, 887–896. https://doi.org/10.1007/s12639-021-01376-z

Gobierno de Canarias, Consejería de Agricultura, Ganadería y Pesca, 2023. https://www.gobiernodecanarias.org/pesca/

Iglesias, R., Rangel, L.F., Fernández-Vázquez, F., Santos, M.J., García-Estévez, J.M., 2022. Morphometric and molecular characterization of Kudoa encrasicoli n. sp. (Myxozoa: Myxosporea) from the European anchovy, Engraulis encrasicolus (L.) (Clupeiformes: Engraulidae). Syst Parasitol 99, 621–636. https://doi.org/10.1007/s11230-022-10051-7

Moran, J.D.W., Whitaker, D.J., Kent, M.L., 1999. A review of the myxosporean genus Kudoa Meglitsch, 1947, and its impact on the international aquaculture industry and commercial fisheries, Aquaculture.

Martínez De Velasco, G., Rodero, M., Chivato, T., Cuéllar, C., 2007. Seroprevalence of anti-Kudoa sp. (Myxosporea: Multivalvulida) antibodies in a Spanish population. Parasitol Res 100, 1205–1211. https://doi.org/10.1007/s00436-006-0390-x

Morozińska-Gogol, J., 2019. Anisakis spp. as etiological agent of zoonotic disease and allergy in European region – an overview. Ann Parasitol. https://doi.org/10.17420/ap6504.214

Partearroyo, T., Samaniego-Vaesken, M. de L., Ruiz, E., Aranceta-Bartrina, J., Gil, Á., González-Gross, M., Ortega, R.M., Serra-Majem, L., Varela-Moreiras, G., 2019. Current food consumption amongst the spanish anibes study population. Nutrients 11. https://doi.org/10.3390/nu11112663

Paolacci, S., Mendes, R., Klapper, R., Velasco, A., Ramilo-Fernandez, G., Muñoz-Colmenero, M., Potts, T., Martins, S., Avignon, S., Maguire, J., De Paz, E., Johnson, M., Denis, F., Pardo, M.A., McElligott, D., Sotelo, C.G., 2021. Labels on seafood products in different European countries and their compliance to EU legislation. Mar Policy 134. https://doi.org/10.1016/j.marpol.2021.104810

Rodriguez-Ponce, E., Molina ', J.M., Hernhdez, S., 1995. Seroprevalence of goat toxoplasmosis on Grand Canary Island (Spain), PREVENTIVE VETERINARY MEDICINE.

Rodríguez-Ponce, E., Betancor, E., Steinhagen, D., Ramírez, A.S., Ventura, M.R., de Felipe, M.C., Pestano, J., 2019. Kudoa sp. (Myxozoa, Multivalvulida): first report in five commercial fish species from the Canary Islands-FAO 34 (Macaronesia-Spain). Parasitol Res 118, 2567–2574. https://doi.org/10.1007/s00436-019-06415-6

Rubio, C., Gutiérrez, Burgos, A., Hardisson, A., 2008. Total dietary intake of mercury in the canary islands, Spain. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 25, 946–952. https://doi.org/10.1080/02652030801993597

Schroeder, H.W., Cavacini, L., 2010. Structure and function of immunoglobulins. Journal of Allergy and Clinical Immunology 125. https://doi.org/10.1016/j.jaci.2009.09.046

Sharp, M.F., Lopata, A.L., 2014. Fish allergy: In review. Clin Rev Allergy Immunol. https://doi.org/10.1007/s12016-013-8363-1

Sugita-konishi, Y., Sato, H., Ohnishi, T., 2014. Novel Foodborne Disease Associated with Consumption of Raw Fish, Olive Flounder (Paralichthys olivaceus). Food Safety 2, 141–150. https://doi.org/10.14252/foodsafetyfscj.2014026

Shin, S.P., Shirakashi, S., Hamano, S., Kato, K., Lasso, L.T., Yokoyama, H., 2016. Phylogenetic study of the genus Kudoa (Myxozoa: Multivalvulida) with a description of Kudoa rayformis sp. nov. from the trunk muscle of Pacific sierra Scomberomorus sierra. Mol Phylogenet Evol 98, 337–345. https://doi.org/10.1016/j.ympev.2016.02.019

Williams, M., Hernandez-Jover, M., Shamsi, S., 2020. Fish substitutions which may increase human health risks from zoonotic seafood borne parasites: A review. Food Control. https://doi.org/10.1016/j.foodcont.2020.107429