 doi:10.20944/preprints202208.0437.v1

*Article*

# Evolution of COVID-19 Spread in the Cohort of LaLiga Football (Soccer) Professionals between May 2020 and April 2021

**Marina Peñuelas[1], Ayelen Rojas[1,2], María Guerrero-Vadillo[1], Inmaculada León-Gómez[1,2], Amparo Larrauri[1,2], Francecs Prats[3], Antonio Fuertes[3], Rafael Zambrano-López[4], Rebeca Ramis[1,2] and Diana Gomez-Barroso[1,2,*]**

[1] National Centre for Epidemiology, Instituto de Salud Carlos III (ISCIII), Madrid, Spain
[2] Consortium for Biomedical Research in Epidemiology and Public Health (CIBERESP), Madrid, Spain
[3] Synalb Diagnósticos Globales S.A., Barcelona, Spain
[4] LaLiga, Madrid
*Corresponding author: dgomez@isciii.es

**Abstract:** Objectives: COVID-19 pandemic interrupted the Spanish professional football competition until May 2020, when it was restarted following a surveillance protocol established by LaLiga. The aims were to describe the infective and serological status of professional football players (PLY) and staff (STF) between May 5th 2020 until April 22nd 2021, to analyze the spatial-temporal distribution of the COVID-19 disease in this cohort and its comparison to the Spanish population. Methods: a prospective observational cohort study was carried out. Differences between PLY and STF were assessed by Chi-squared test and test of equality of proportions. Pearson correlation test was used to measure the presence of an association between the percentages of positivity in population and LaLiga cohort. Results: 137,420 RT-PCR and 20,376 IgG serology tests were performed in 7,112 professionals. Positive baseline serology was detected in 10.57% of PLY and 6.38% of STF. Among those who started the follow-up as not infected and before STF vaccination, 11.87% of PLY and 5.03% of STF became positive. Before summer 2020 the prevalence of infection was similar than the observed at national level. The percentage of positivity in the Spanish population was higher than in LaLiga cohort, but both series showed a similar decreasing trend.

**Keywords:** COVID-19; LaLiga; sports; spatial and temporal analysis; serological status

## 1. Introduction

LaLiga is a global, innovative and socially responsible organisation, a leader in the leisure and entertainment sector in Spain. It is a private law Sports Association, which according to Spanish law is solely and obligatorily comprised of all the Sports Corporations and Clubs that take part in official nation-wide professional football competitions (LaLiga Santander & LaLiga Smartbank). [1].

In Spain, the football season 19/20 was interrupted by the lockdown imposed on 14th March [2,3] following the emergence of the COVID-19 pandemic. On May 2020, the competition was reopened although the risk of SARS-CoV-2 transmission among professional football players was unknown, and knowledge of the disease effects in athletes was limited [4]. In this situation, LaLiga in collaboration with the sports authorities and medical team, established specific guidelines for the resumption of professional competitions, reported in a COVID-19 Protocol with the aim of ensuring the return to competition and sporting activity with the maximum health and safety guarantees. These guidelines were agreed considering the health status of the athletes after confinement, the reduction of the likelihood of COVID-19 infection during training and competition, and the development of injury prevention strategies [2,5]. This strategy changed over time, adapting to the epidemiological situation in the country and included actions such as a COVID-19 monitoring strategy, the implementation of extensive mitigation and surveillance measures,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 26 August 2022 doi:10.20944/preprints202208.0437.v1

including non-pharmacological measures and frequent routine testing, with around 262,000 antigen tests, 140,000 PCR tests and 15,000 serologies[2,5].

The aims of this study were to describe the infective and serological status of professional football players (PLY) and staff (STF) after the restart of the competition on May 5th 2020 until April 22nd 2021, to analyze the spatial-temporal distribution of the COVID-19 disease in this cohort and its comparison to the Spanish population.

## 2. Materials and Methods

### *2.1. Study design*

This is a prospective cohort observational study. We studied a cohort formed by a football professional PLY and STF from 20 clubs in first division and 22 in second division. The football PLY are men between 18-40 years. STF are mostly men between 18-65 years. Figure 1 show the spatial distribution of cities with one or more football clubs on the competition.

**Figure 1.** Spatial distribution of LaLiga cohort subjects.

The epidemiological variables of participants were: professional category (PLY or STF), city, date of diagnosis, type of test (reverse transcription real-time polymerase chain reaction (RT-PCR) or IgG serology) and testing result.

### *2.2. Monitoring strategy*

From May 2020 to December 2020, encompassing season 2019/2020 and partially 2020/2021, after every long break RT-PCR and serology were performed for every PLY and STF. Before each match a RT-PCR was done 48 hours prior. In January 2021, the pre-match RT-PCR tests were replaced by antigenic test (Ag-test), but if a positive result was obtained, a RT-PCR was performed to confirm the result (Figure 2).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 26 August 2022 doi:10.20944/preprints202208.0437.v1

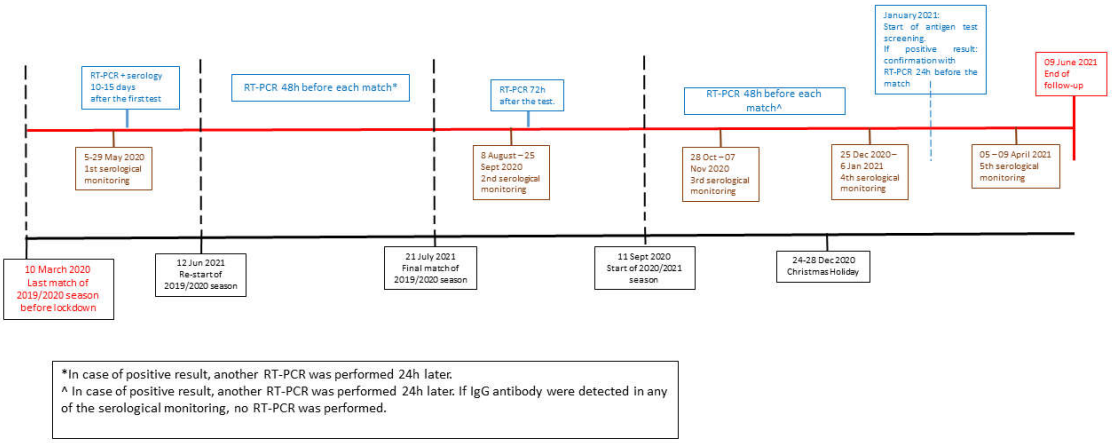


**Figure 2.** Timeline of LaLiga COVID-19 monitoring strategy from May 5th 2020 to April 22nd 2021.

If a person yielded a positive result, it would be isolated and no further testing will be done until the next match. Whereas if >2 members of the same team ("bubble group") tested positive, RT-PCR were mandatory for all members of the "bubble group" involved. In addition, those PLY who played for their national teams or international championships were also tested by RT-PCR and serology upon they return. Not all PLY and STF of each team were tested before each match, but only those who were going to participate in the match. However, non-participants were tested whenever the team requested it.

Throughout the entire period, five serological controls independent of the previous results were performed to every PLY and STF to assess changes in the seroepidemiology of the cohort. If a positive result was obtained, subsequent tests were serological, replacing pre-match RT-PCR or Ag-tests by a serological one. However, these serological tests were not marked, so they could not be identified among all serologies. Only RT-PCR and serology results were included in the study. All laboratory test were performed by the same company (Synlab Diagnósticos Globales®) following the same procedures.

### *2.3. RT-PCR testing*

Nasopharyngeal samples were used for molecular diagnosis. First, RNA extraction was performed using the KingFisher instrument and MagMAX™ Pathogen RNA/DNA Kit assay (ThermoFisher Scientific, USA). Then, the TaqPathTM COVID-19 assay (ThermoFisher Scientific, USA) carried out in QuantStudio 5 thermocyclers was performed for amplification and detection. This assay detects three gene targets from the ORF1a/b, S, and N regions of Sars-CoV-2. Ct (cycle threshold) was not reported. Results were considered as positive or negative according to manufacturer's instructions.

### *2.4. Serological testing*

For IgG detection, until approximately week 44/2020, it was used a qualitative chemiluminescent microparticle immunoassay (CMIA) detecting SARS-CoV-2 IgG against nucleoparticle (SARS-CoV-2 Abbott®; cut off: 1,4), when it was replace by other quantitative chemiluminescent immunoassay (CLIA) detecting IgG against S1 and S2 spike proteins (LIAISON ® SARS-CoV-2 S1/S2 IgG; cut off: 15 UA/ml). In 42 determinations between week 35/2020 and week 10/2021 it was used an ELISA test detecting IgG against S1 protein (Anti-SARS-CoV-2 ELISA (IgG) Euroimmune®; cut off: 1,1). For seroepidemiological analyses, all serological results were considered qualitative.

As the vaccination of healthcare workers (HCW) started in early 2021, positive serological results of STF after February 1st were not taken into account or otherwise it was indicated. Only subjects with >1 test were included for the assessment of IgG gain and loss. Even if there are not reference data to decide when IgG loss is trusted, less than 60

 doi:10.20944/preprints202208.0437.v1

days seems to reflect a false result [6,7], however, as we were not aware of the clinical interpretation of these tests, as well as information about comorbidities and other possible explanations, we decided to include all results.

According to the five serological control dates, we selected IgG tests performed in those periods to measure the seroepidemiology evolution with two different approaches depending on restrictiveness. The most restrictive approach considers a control result as positive only if all the results included in that control period were positive; and the less restrictive approach considers a control result as positive if any of the test included in that control period were positive. Only those subjects with results in all controls were included in this evaluation.

### *2.5. Statistical analysis*

The following data were calculated: median and interquartile range for the number of each test, percentage of IgG positive subjects at the beginning of the follow-up, percentage of subjects who become positive among those who started the follow-up period as not infected (IgG negative), percentage of subjects who lost IgG and percentage of those who become positive again. Differences between PLY and STF were assessed by Chi-squared test and test of equality of proportions. Statistical significance was defined as p-value < 0.05. Analysis were carried out using Stata, R and Excel.

### *2.6. Spatio-temporal analysis*

Percentage of positivity by month and city were calculated for LaLiga cohort and for the Spanish general population. We divided the number of positive RT-PCR and the total number of RT-PCR tests carry out per month and city. For the general population we used RT-PCR and Ag-tests results from the Laboratory Results Registry (SERLAB) to obtain the percentage of COVID-19 positivity in Spain. Only tests carried out on the population aged 15-65 have been taken into account. Using Shiny R package we created an interactive map with the spatio-temporal evolution of the COVID-19 positivity.

Pearson correlation test was used to measure the presence of an association between the percentages of positivity in Spanish population and LaLiga cohort.

## 3. Results

Between 5th May 2020 (week 18/2020) and 22nd April 2021 (week 16/2021), 7112 football professionals underwent tests for current or past Covid-19 infection. During this period were performed a total of 137,420 RT-PCR and 20,376 serology tests for IgG detection. Therefore, 98% subjects underwent RT-PCR testing and 82.31% underwent serological testing. Table 1 shows the distribution of test and results in PLY and STF.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 26 August 2022 doi:10.20944/preprints202208.0437.v1

**Table 1.** Classification of the players and staff members who underwent RT-PCR and/or serology tests between 5th May 2020 and 22nd April 2021 according to their results.

| | Total number of subjects (%) | | | |
|---|---|---|---|---|
| **Status** | **Total** | **Players** | **Staff** | **Comments** |
| **RT-PCR only** | 1,258 | 228 (18.12) | 1,030 (81.88) | |
| *All RT-PCR negative* | 1,241 (98.65) | 222 (97.37) | 1,019 (98.93) | |
| *≥1 RT-PCR positive* | 17 (1.35) | 6 (2.63) | 11 (1.07) | |
| **Serology only** | 142 | 71 (50) | 71 (50) | |
| *One serology. IgG+* | 42 (29.58) | 15 (21.13) | 27 (38.03) | Including vaccinated staff. |
| *One serology. IgG-* | 68 (47.89) | 32 (45.07) | 36 (50.70) | |
| *>1 serology. ≥1 IgG+* | 31 (21.83) | 23 (32.39) | 8 (11.27) | Including vaccinated staff. |
| *All IgG negative* | 1 (0.70) | 1 (1.41) | 0 | |
| **Both RT-PCR and serology** | 5712 | 2,191 (38.36) | 3,521 (61.64) | |
| *One RT-PCR and one IgG performed the same day* | 179 (3.13) | 39 (1.78) | 140 (3.98) | 164 RT-PCR and IgG negative; 12 only IgG positive; 3 RT-PCR and IgG positive |
| *All RT-PCR and IgG negative* | 4,312 (75.49) | 1,552 (70.84) | 2,760 (78.34) | Only 1 serology in 288 PLY and 799 STF. |
| *RT-PCR+ without subsequent serology (all previous IgG were -)* | 36 (0.63) | 23 (1.05) | 13 (0.36) | Only 1 serology in 1 PLY and 7 STF. |
| *RT-PCR+ before the first IgG+* | 381 (6.67) | 227 (10.36) | 154 (4.37) | Only 1 serology in 5 PLY and 5 STF. |
| *RT-PCR+ after the first IgG+* | 31 (0.54) | 22 (1.00) | 9 (0.26) | Only 8 RT-PCR+ >90 days after first IgG+. 1 IgG in 1 STF. |
| *RT-PCR+ without seroconversion* | 87 (1.52) | 42 (1.92) | 45 (1.28) | Only 1 serology in 8 STF. |
| *RT-PCR- but IgG+* | 686 (12.01) | 286 (13.05) | 400 (11.36) | Including vaccinated staff. Only 1 serology in 31 PLY and 26 STF. |
| **Total** | 7,112 | 2,490 (35.01) | 4,622 (64.99) | |

Both groups underwent a median of 22 and 12 RT-PCR respectively (IQR=34, range 1-60 for PLY and IQR=25, range 1-58 for STF). Only 7.68% of the PLY and 11.07% of STF underwent a single RT-PCR. At least one positive RT-PCR was detected in 320 (13.23%) PLY and 235 (5.16%) STF, and among those, time and number of test until negative result were available for 75% of PLY and 69.79% of STF (Table 2). Twelve PLY and 12 STF tested negative in the first 48 hours after testing positive. In the other hand, 24 PLY and 12 STF, SARS-CoV-2 remained detectable in five or more RT-PCR after the first positive result, with a positivity duration of more than 3 weeks. Thirty of these cases of prolonged RT-PCR positivity occurred between July and October 2020.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 26 August 2022 doi:10.20944/preprints202208.0437.v1

**Table 2.** Number of players and staff members with a positive RT-PCR classified by days and number of RT-PCR tests until the next negative result between 5th May 2020 and 22nd April 2021.

| | Number of RT-PCR test to NEGATIVE | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | ≥5 | | Total |
| | PLY | STF | PLY | STF | PLY | STF | PLY | STF | PLY | STF | |
| Days to NEG | | | | | | | | | | | |
| 0 | 1 | 1 | | | | | | | | | 2 |
| 1-2 | 11 | 11 | | | | | | | | | 22 |
| 3-5 | 19 | 17 | | | | | | | | | 36 |
| 6-10 | 14 | 16 | 16 | 6 | 3 | 1 | | | | | 56 |
| 11-15 | 17 | 14 | 17 | 15 | 11 | 3 | 8 | 3 | 5 | | 93 |
| 16-21 | 8 | 3 | 8 | 10 | 20 | 10 | 12 | 1 | 8 | 2 | 82 |
| 22-28 | 5 | 5 | 2 | 2 | | 1 | 4 | 1 | 13 | 7 | 40 |
| ≥29 | 10 | 6 | 7 | 15 | 6 | 7 | 4 | 2 | 11 | 5 | 73 |
| Total | 85 | 73 | 50 | 48 | 40 | 22 | 28 | 7 | 37 | 14 | 404 |

PLY: Players; STF: Staff members; NEG: Negative result.

Regarding serology, PLY and STF underwent a median of 4 (IQR=4, range 1-19) and 3 tests (IQR=4, range 1-14) respectively throughout the entire period. For 18.17% of PLY and 29.20% of STF only one serology was performed. Time of serological follow-up, among those who underwent more than one test, showed a high variation, getting a median of 239 days (IQR=236, range 3-51) for PLY, and 234 days (IQR=236, range 0-341) for STF. According to the diagnosis strategy, those who tested positive gathered the higher number of serological test. Those who became negative again after one or more IgG positive results underwent further testing (p-value<0.0001), as well as those who returned positive again afterwards (p-value=0.0003).

At the beginning of the follow-up (date of first IgG test per individual), a result compatible with a past infection (IgG positive) were detected in 10.57% of PLY and 6.38% of STF, being significantly higher in PLY (p-value<0.0001). Between those with positive baseline serology and RT-PCR follow-up, 1.5% of subjects (5 PLY and 2 STF) got a positive RT-PCR more than 90 days after baseline serology. In the other hand, among those who started the follow-up as not infected and up to February 1st (before STF vaccination), 11.87% of PLY and 5.03% of STF became positive. By the end of the study period 16.66% of PLY had become positive. In this group of not pre-infected, only one PLY got a positive RT-PCR more than 90 days after the first IgG positive result, when IgG was negative again.

Antibody loss was detected in 22.03% of PLY and 13.73% of STF, with differences between them (p-value<0.001). Among them, 29.57% of PLY and 36.49% of STF recovered IgG subsequently, with several IgG losses and gains evidences in 3 PLY. Two thirds of STF recovered IgG before February 1st. Absolute seroreversion (IgG loss without subsequent recovery) was detected in 12% of subjects with differences between PLY and STF (15.51% vs. 8.72%, respectively, p-value<0.001). More than half of subjects (61.398%) who lost IgG started the study as seropositive, but in 50 PLY and 23 STF dates of first positive result and first subsequent negative result were recorded. The median number of tests performed between these dates was 1 for both groups (IQR=1, range 1-4 for PLY and 1-7 for STF), meaning a median of 92.5 days (IQR=103, range 3-240) for PLY and 69 days (IQR=112, range 0-204) for STF. On the other hand, the number of tests between IgG loss and recovery showed a median of 1 test for both groups (IQR=0, range 1-4), and a median of 62 days (IQR=35, range 15-212) for PLY and 75 days (IQR=29, range 29-156) for STF.

Among the 5854 subjects who underwent serological test, 1,311 (22.39%) had results for the 5 serological controls (Table 3): 641 PLY and 670 STF. The last control includes results of STF once vaccination had begun.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 26 August 2022 doi:10.20944/preprints202208.0437.v1

**Table 3.** Number and percentage of positivity in LaLiga five serological controls by professional category.

| | Less restrictive strategy | | | More restrictive strategy | | | ROUND Dates | |
|---|---|---|---|---|---|---|---|---|
| Round | ALL N (%) | PLY N (%) | STF N (%) | ALL N (%) | PLY N (%) | STF N (%) | Start date | Finish date |
| 1 | 78 (5.95) | 38 (5.93) | 40 (5.97) | 67 (5.11) | 31 (4.84) | 36 (5.37) | 05/may/20 | 29/may/20 |
| 2 | 106 (8.09) | 67 (10.45) | 39 (5.82) | 74 (5.64) | 42 (6.55) | 32 (4.78) | 08/aug/20 | 25/sep/20 |
| 3 | 147 (11.21) | 98 (15.29) | 49 (7.31) | 143 (10.91) | 96 (14.98) | 47 (7.01) | 28/oct/20 | 07/nov/20 |
| 4 | 189 (14.19) | 118 (18.41) | 68 (10.15) | 184 (14.04) | 116 (18.10) | 68 (10.15) | 25/dec/20 | 06/jan/21 |
| 5 | 330 (25.17) | 146 (22.78) | 184 (27.46) | 324 (24.71) | 141 (22) | 183 (27.31) | 05/apr/21 | 09/apr/21 |

PLY=players, STF=Staff.

Regarding spatial-temporal analysis, Table 4 shows the percentage of positivity in LaLiga cohort and the Spanish population by month. The positivity in the Spanish population was much higher than in LaLiga cohort, but both series showed a similar decreasing trend with the Pearson correlation of 0.28. If June 2020 was not account, the correlation increased to 0.86. This application https://coviddifusion.isciii.es/positivLaLiga/ shows the spatio-temporal evolution of the percentage of positivity by month and city in LaLiga cohort and in general population. The Graphic compares the result of both populations over time.

**Table 4.** Percentage of positivity by month for all players and staff members between 5th May 2020 and 22nd April 2021.

| Month | % positivity LaLiga | % positivity Spain |
|---|---|---|
| 01/04/2020 | 0.01 | 0.17 |
| 01/05/2020 | 0.15 | 2.98 |
| 01/06/2020 | 0.03 | 11.96 |
| 01/07/2020 | 0.25 | 4.51 |
| 01/08/2020 | 0.16 | 3.47 |
| 01/09/2020 | 0.04 | 3.08 |
| 01/10/2020 | 0.06 | 1.65 |
| 01/11/2020 | 0.06 | 1.81 |
| 01/12/2020 | 0.08 | 1.2 |
| 01/01/2021 | 0.04 | 0.49 |
| 01/02/2021 | 0.02 | 0.06 |
| 01/03/2021 | 0.07 | 0.36 |
| 01/04/2021 | 0.02 | 0.14 |
| 01/05/2021 | 0.01 | 0.05 |
| 01/06/2021 | 0 | 0 |

## 4. Discussion

This cohort study, based on LaLiga PLY and STF, shows the results of COVID-19 monitoring strategy between May 2020 and April 2021. The strategy followed by LaLiga was similar to those followed in other countries and for different close-contact sports [8,9].

Of the 7,112 subjects tested, LaLiga's follow-up strategy probably detected between 434 and 555 (including positive IgG obtained before or at the same time as the positive RT-PCR) subjects with the capacity to infect others, which represents between 6% and 7.8% of total subjects in the cohort for the whole period. To avoid data skewing, since critical information like Ct values for RT-PCR tests was not available, every result was taken into account. Unusual results, e.g. opposite results in less than 48 hours, were also described in other leagues following a similar tracking strategy [9,10] and is normal when

 doi:10.20944/preprints202208.0437.v1

mass testing was carried out independently of symptoms and epidemiological information and when the prevalence of infection is low [11].

In the other hand, 24 PLY and 12 STF had ≥5 positive RT-PCR along more than 21 days. Although no information about symptomatology was available, it can be assumed that they were re-tested because they had already recovered and were ready to play a match. Prolonged viral shedding after symptoms resolution and seroconversion was initially reported in April 2020 [12] with further reports in the following months [13,14], and from October 2020 ECDC updated its recommendations to end the isolation of asymptomatic and mild cases, from a test-based to a symptom-based strategy [15]. Adaptation of this recommendation to LaLiga strategy can explain why cases with prolonged viral shedding were mostly detected in summer 2020.

The high number of serological tests performed (and consequently the longer follow-up time) among PLY is explained by the high number of PLY with a positive baseline result. In our series, 12% of participants with IgG against SARS-CoV-2 and more than 1 test performed presented seroreversion. Several studies reported loss of antibodies over time: IgG loss of 28.2% was observed in a 60-day follow-up study (April-June 2020) among HCW in USA [16], while in another study among HCW in Chicago, the percentage of seronegativization among those participants with positive IgG at the beginning of a 6-months follow-up (May-June 2020) was 52%.[17]. Early IgG losses and, in some cases also IgG recoveries, are difficult to explain in absence of a clinical and epidemiological background data. Some results are almost impossible from a biological point of view, for instance, IgG loss < 30 days after first detection. In addition, no neutralization test were performed in any seroreversion to confirm them. These atypical results are probably due to random errors which can occur from the sample collection to the result releasing, including test limitations. It must be taken into account that serology is an indirect technique, usually more susceptible to cross-reactions and less specific than direct techniques as PCR [18]. All these techniques were developed and validated in a global health emergency, which probably had an impact on their sensitivity and specificity [19–21]. Most of the atypical results were obtained during the first half of the period, so the lack of sensitivity and specificity of the techniques applied seems to be the most reliable explanation. In a similar but shorter follow-up cohort after the restart of the German football league (Bundesliga) none of seroreverse cases was confirmed [22]. Furthermore, a higher number of tests are statistically linked to a higher number of atypical results, as occurred in our series. In any case, we would like to reinforce the idea that laboratory results in the absence of clinical and epidemiological data are clearly susceptible to misinterpretation, reducing their quality, and must be avoided.

Considering reinfection as those cases with a positive RT-PCR >90 days after the first positive IgG, only 8 cases met the criteria (0.14% of those with PCR and serology results and 1.5% when counting only those subjects with seropositive baseline status), being much more lower than those detected among HCW, 2.5% [17], using the same reinfection definition.

Seroepidemiological controls showed that before summer 2020 the prevalence of infection was similar than the observed in the national seroepidemiological study for all age groups, around 5-5.2% [23], whereas in November 2020 the national estimated prevalence was around 9.9% which is higher than observed between STF, around 7.3%, but lower than observed in PLY, around 15%. However, at that moment most of the COVID-19 cases were diagnosed in people between 15 and 29 years old [24], the same age group than most of the PLY. The wide age range of STF avoids its comparison to any age group. It seems that the seroepidemiological evolution of PLY was similar to the expected for their age group in Spain. The lower seroprevalence of COVID-19 in STF compared with the national value, could be explained by a higher level of awareness and compliance with LaLiga's recommendations, as proposed in a study involving the National Football League (American football), which detected a lower incidence rate than the estimated in the surrounding community during a 5 months period and linked it with their strict protocols [25]. However, the trend of RT-PCR percentage of positivity in LaLiga is correlated

 doi:10.20944/preprints202208.0437.v1

with that obtained at national level from the summer 2020 to the end of the study period, and as diagnostic test became more widely available in general population this correlation seems to be higher.

This study has several limitations, starting by the lack of basic demographic information on sex and age, as well as relevant clinical and epidemiological information such as the presence and severity of symptoms, history of recent close contact with a case, travel background, or the clinical interpretation of laboratory results. As all PLY were male, our results cannot be extrapolated to female athletes. Football club origin for each subject was unavailable and the follow-up strategy was not consistent for the entire data period, being these two of the major limitations for the spatial-temporal analysis. To partially solve this problem data were aggregated by month. National data used in this study have also several limitations due to the differences in testing strategy and reporting capacity between regions.

## 5. Conclusion

LaLiga cohort is probably the largest studied cohort in terms of number of subjects and time of follow up. During the study period and with the available data, we could detect neither clusters nor moments of high risk of infection related to football matches, which could probably be partly explained by LaLiga's monitoring strategy, and thus could be considered a successful strategy in preventing outbreaks after football matches.

Furthermore, when comparing similarities between LaLiga and national seroepidemiological data, as well as the correlation of RT-PCR positivity percentage along time, we see that both, LaLiga cohort trend and the national trend, followed the same pattern, what have been observed in other studies too [26,27]. From an epidemiological point of view, the trend of the pandemic spread did not change in this cohort.

**Author Contributions:** Diana Gómez-Barroso and Rebeca Ramis conceived the original idea and were in charge or overall planning. Inmaculada León-Gómez, Marina Peñuelas, Ayelén Rojas, María Guerrero and Rebeca Ramis analysed the data. Amparo Larrauri contributed to the design of the study and the interpretation of the results. Ayelén Rojas, María Guerrero-Vadillo and Diana Gómez-Barroso designed the figures. Marina Peñuelas and Rebeca Ramis designed the tables. Francesc Prats and Antonio Fuertes helped to interpret the laboratory results and contributed to the drafting. Marina Peñuelas and Diana Gómez-Barroso wrote the article. Rafael Zambrano ,María Guerrero-Vadillo, Ayelén Rojas and Rebeca Ramis reviewed preliminary drafts. All authors discussed the results and contributed to the final version of the manuscript.

**Informed Consent Statement:** Not applicable.

**Acknowledgments:** We wish to acknowledge the great work carried out by all the staff of Synlab Laboratoires. We would also like to give special thanks to Jaime Blanco from LaLiga, who made this work possible.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Página web oficial de LaLiga Available online: https://www.laliga.com/ (accessed on 15 December 2021).
2. Brito de Souza, D.; López-Del Campo, R.; Resta, R.; Moreno-Perez, V.; Del Coso, J. Running Patterns in LaLiga Before and After Suspension of the Competition Due to COVID-19. *Front Physiol* **2021**, *12*, 666593, doi:10.3389/fphys.2021.666593.
3. BOE.Es - BOE-A-2020-3692 Real Decreto 463/2020, de 14 de Marzo, Por El Que Se Declara El Estado de Alarma Para La Gestión de La Situación de Crisis Sanitaria Ocasionada Por El COVID-19. Available online: https://www.boe.es/buscar/act.php?id=BOE-A-2020-3692 (accessed on 15 December 2021).
4. Phelan, D.; Kim, J.H.; Chung, E.H. A Game Plan for the Resumption of Sport and Exercise After Coronavirus Disease 2019 (COVID-19) Infection. *JAMA Cardiol* **2020**, *5*, 1085, doi:10.1001/jamacardio.2020.2136.
5. Herrero-Gonzalez, H.; Martín-Acero, R.; Del Coso, J.; Lalín-Novoa, C.; Pol, R.; Martín-Escudero, P.; De la Torre, A.I.; Hughes, C.; Mohr, M.; Biosca, F.; et al. Position Statement of the Royal Spanish Football Federation for the Resumption of Football Activities after the COVID-19 Pandemic (June 2020). *Br J Sports Med* **2020**, *54*, 1133–1134, doi:10.1136/bjsports-2020-102640.

6. Adams, E.R.; Ainsworth, M.; Anand, R.; Andersson, M.I.; Auckland, K.; Baillie, J.K.; Barnes, E.; Beer, S.; Bell, J.I.; Berry, T.; et al. Antibody Testing for COVID-19: A Report from the National COVID Scientific Advisory Panel. *Wellcome Open Res* **2020**, *5*, 139, doi:10.12688/wellcomeopenres.15927.1.
7. Kim, D.S.; Rowland-Jones, S.; Gea-Mallorquí, E. Will SARS-CoV-2 Infection Elicit Long-Lasting Protective or Sterilising Immunity? Implications for Vaccine Strategies (2020). *Front Immunol* **2020**, *11*, 571481, doi:10.3389/fimmu.2020.571481.
8. Mack, C.D.; Wasserman, E.B.; Perrine, C.G.; MacNeil, A.; Anderson, D.J.; Myers, E.; Smith, S.; McDonald, L.C.; Osterholm, M.; Solomon, G.S.; et al. Implementation and Evolution of Mitigation Measures, Testing, and Contact Tracing in the National Football League, August 9-November 21, 2020. *MMWR Morb Mortal Wkly Rep* **2021**, *70*, 130–135, doi:10.15585/mmwr.mm7004e2.
9. Rankin, A.; Massey, A.; Falvey, É.C.; Ellenbecker, T.; Harcourt, P.; Murray, A.; Kinane, D.; Niesters, B.; Jones, N.; Martin, R.; et al. Infographic. COVID-19 RT-PCR Testing for Elite Athletes. *Br J Sports Med* **2021**, *55*, 818–820, doi:10.1136/bjsports-2020-103751.
10. Hassanmirzaei, B.; Haratian, Z.; Ahmadzadeh Amiri, A.; Ahmadzadeh Amiri, A.; Moghadam, N. SARS-CoV-2 Serological Assay and Viral Testing: A Report of Professional Football Setting. *Postgrad Med J* **2021**, postgradmedj-2021-140176, doi:10.1136/postgradmedj-2021-140176.
11. Grunér, M.; Nordberg, M.; Lönnroth, K. Problems Associated with Mass Border Testing of COVID-19. *Scand J Public Health* **2022**, *50*, 22–25, doi:10.1177/14034948211023659.
12. Liu, W.-D.; Chang, S.-Y.; Wang, J.-T.; Tsai, M.-J.; Hung, C.-C.; Hsu, C.-L.; Chang, S.-C. Prolonged Virus Shedding Even after Seroconversion in a Patient with COVID-19. *J Infect* **2020**, *81*, 318–356, doi:10.1016/j.jinf.2020.03.063.
13. Li, N.; Wang, X.; Lv, T. Prolonged SARS-CoV-2 RNA Shedding: Not a Rare Phenomenon. *J Med Virol* **2020**, *92*, 2286–2287, doi:10.1002/jmv.25952.
14. Xiao, A.T.; Tong, Y.X.; Zhang, S. Profile of RT-PCR for SARS-CoV-2: A Preliminary Study From 56 COVID-19 Patients. *Clinical Infectious Diseases* **2020**, *71*, 2249–2251, doi:10.1093/cid/ciaa460.
15. European Centre for Disease Prevention and Control. *Guidance for Discharge and Ending Isolation of People with COVID-19, 16 October 2020.*; Stockholm: ECDC, 2020;
16. Self, W.H.; Tenforde, M.W.; Stubblefield, W.B.; Feldstein, L.R.; Steingrub, J.S.; Shapiro, N.I.; Ginde, A.A.; Prekker, M.E.; Brown, S.M.; Peltan, I.D.; et al. Decline in SARS-CoV-2 Antibodies After Mild Infection Among Frontline Health Care Personnel in a Multistate Hospital Network - 12 States, April-August 2020. *MMWR Morb Mortal Wkly Rep* **2020**, *69*, 1762–1766, doi:10.15585/mmwr.mm6947a2.
17. Wilkins, J.T.; Hirschhorn, L.R.; Gray, E.L.; Wallia, A.; Carnethon, M.; Zembower, T.R.; Ho, J.; DeYoung, B.J.; Zhu, A.; Rasmussen-Torvik, L.J.; et al. Serologic Status and SARS-CoV-2 Infection over 6 Months of Follow Up in Healthcare Workers in Chicago: A Cohort Study. *Infect Control Hosp Epidemiol* **2021**, 1–9, doi:10.1017/ice.2021.367.
18. Fierz, W. Basic Problems of Serological Laboratory Diagnosis. In *Molecular Diagnosis of Infectious Diseases*; Humana Press: New Jersey, 2003; Vol. 0, pp. 393–428 ISBN 978-1-59259-679-9.
19. Sidiq, Z.; Hanif, M.; Dwivedi, K.K.; Chopra, K.K. Benefits and Limitations of Serological Assays in COVID-19 Infection. *Indian J Tuberc* **2020**, *67*, S163–S166, doi:10.1016/j.ijtb.2020.07.034.
20. West, R.; Kobokovich, A.; Connell, N.; Gronvall, G.K. COVID-19 Antibody Tests: A Valuable Public Health Tool with Limited Relevance to Individuals. *Trends Microbiol* **2021**, *29*, 214–223, doi:10.1016/j.tim.2020.11.002.
21. Gong, F.; Wei, H.; Li, Q.; Liu, L.; Li, B. Evaluation and Comparison of Serological Methods for COVID-19 Diagnosis. *Frontiers in Molecular Biosciences* **2021**, *8*.
22. Meyer, T.; Mack, D.; Donde, K.; Harzer, O.; Krutsch, W.; Rössler, A.; Kimpel, J.; Laer, D. von; Gärtner, B.C. Successful Return to Professional Men's Football (Soccer) Competition after the COVID-19 Shutdown: A Cohort Study in the German Bundesliga. *Br J Sports Med* **2021**, *55*, 62–66, doi:10.1136/bjsports-2020-103150.
23. Pollán, M.; Pérez-Gómez, B.; Pastor-Barriuso, R.; Oteo, J.; Hernán, M.A.; Pérez-Olmeda, M.; Sanmartín, J.L.; Fernández-García, A.; Cruz, I.; Fernández de Larrea, N.; et al. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): A Nationwide, Population-Based Seroepidemiological Study. *Lancet* **2020**, *396*, 535–544, doi:10.1016/S0140-6736(20)31483-5.
24. Red Nacional de Vigilancia Epidemiológica *Situación de COVID-19 En España. Casos Diagnosticados a Partir 10 de Mayo.*; Instituto de Salud Carlos III, 2020;
25. Lopez, M.J.; Mack, C.; Bliss, T.; Wasserman, E.B.; Myers, E.; Solomon, G.; Lynch, J.; Casolaro, A.; Osterholm, M.; Hanfling, D.; et al. Observed Versus Expected COVID-19 Infections Among National Football League Players During the 2020 Season. *Epidemiology* **2021**, doi:10.1097/EDE.0000000000001416.
26. Schumacher, Y.O.; Tabben, M.; Hassoun, K.; Al Marwani, A.; Al Hussein, I.; Coyle, P.; Abbassi, A.K.; Ballan, H.T.; Al-Kuwari, A.; Chamari, K.; et al. Resuming Professional Football (Soccer) during the COVID-19 Pandemic in a Country with High Infection Rates: A Prospective Cohort Study. *Br J Sports Med* **2021**, *55*, 1092–1098, doi:10.1136/bjsports-2020-103724.
27. Gualano, B.; Brito, G.M.; Pinto, A.J.; Lemes, I.R.; Matos, L.D.N.J.; de Sá Pinto, A.L.; Loturco, I.; Coalition SPORT-COVID-19 High SARS-CoV-2 Infection Rate after Resuming Professional Football in São Paulo, Brazil. *Br J Sports Med* **2021**, bjsports-2021-104431, doi:10.1136/bjsports-2021-104431.