This paper describes the effect of an Animal Assisted Activity on the well being of End Stage Renal Patients undergoing dialysis using serum levels of Oxytocin and Serotonin as the measure. The hypothesis is not clearly stated- does an increase in Oxytocin and Serotonin indicate an increase in patient well being. No data such as patient self reports are provided to support the hypothesis. At all levels, detail of the what was done and why it was done is lacking. Confounds such as patient pet ownership, education etc are not considered along with if it is the dog or the dog and activity that is important. The conclusions are overstated given the lack of information.

RE: We thank you for your comment because you gave us the opportunity to better explain our study and to reflect on its weak points.

Simple summary

Line 23: Clarity- "..the establishment of the game size..' I am not sure what is meant by game size

RE: We apologise for the lack of clarity in the manuscript. The sentence was rephrased as follows: “The dog, in particular, due to its ethological characteristics, allows the establishment through the play an active relationship, communication and interaction” (lines 25 to 27 pg 1)

Abstract

1 Introduction:

Line 48: Style- remove the “..in fact..” as it does not add to the reading.

RE: The correction was carried out accordingly.

Line 53: Style- change ..and anxiety, that have a significant.... to “...and anxiety that have a significant negative influence on the self management of their disease that is leads to worsening of their condition ..”

RE: The sentence was rephrased accordingly.

Line 56: Style- change “with the dog” to “with a dog”

RE: The correction was carried out accordingly.

Line 60: Style- the second and third paragraphs as not linked- I don’t know why you are talking about Serotonin and Oxytocin. To make it clearer, consider starting the paragraph with something like: To assess the effect of the AAA, ESRD patient serum serotonin and oxytocin levels were measured. Serotonin (5-HT0 and Oxytocin (OXT) are important modulators....”

RE: The sentence was rephrased accordingly.

Line 62: Discuss these papers references 8-10. Give some details about what others have found.
RE: the sentence was integrated like suggested as follows” These chemical messengers interact in the regulation of emotion-based behaviour. The studies highlight the role of regulation of affiliate behaviours and the role of oxytocin for disorders such as autism and others considered social and stress-related disorders including social phobia, post-traumatic stress disorder and some personality disorders. [35-36-37]” (lines 92 to 96. Pg 2)

Line 62-64: Clarity- the sentence starting: “Furthermore.... ASD (Autism).” Is very long and not clearly written. Suggest revising it.

RE: The sentence was rephrased as follows: “Furthermore, studies conducted on 15q dup mice [38] have indicated that interaction of serotonin-oxytocin through 5-HT1A receptors may play a critical role in the normal development of social behaviour. This leads to hypothesize novel therapeutic strategies in different pathologies, including ASD (autism).” (lines 96 to 99. PP 2/3)

2Materials and Methods

2.1 Patients

Line 69: Clarity- how did you decide they had comparable stage of renal damage. Bloods, medical records, measures of functioning renal mass? We need to know to be able to decide if your subjects were suitable for the study. Did you have any other criteria- what about exclusions? Did they have to only have ESRD or could there be other health issues? Did they have to be undergoing a minimal amount of dialysis? Did you assess them on other factors that may affect their response to AAA- such as education, family background, history of pet owning?

RE: “10 patients (7 men and 3 women) aged between 30 and 50 years with a comparable stage of renal damage and a relational difficulty were enrolled in the study. All patients were chosen by the Kidney Center's Physician and Psychologist. The inclusion criteria were: on clinical record (loss of function of both kidneys over 70%, renal parameters: creatinine of about 8-11 mg / dl and azotemia 40-150 mg / dl (threshold values: creatinine: 1.1-1.2 mg / dl Azotemia: 20-30 mg / dl), treatment cycle (3 times a week duration of 3 hours per session), day and time of treatment, homogeneous socio-demographic characteristics (cultural level, education, family background, absence of dog at home, similar lifestyle), relational difficulty (an important tendency to isolate themselves and to remain silent, with little possibility of dialogue between them or with the medical staff). The Psychologist during the intervention, through a participatory observation, monitored the increase/decrease of their dialogic and behavioral interactions between themselves, the operators involved and the dog. The exclusion criteria were: refuse to participate in the project, allergy, fear of the dog, animals possession at home, other levels of renal damage, concomitant diseases.” (lines 104 to 116. Pg 3)

If you don’t measure these things how do you know if the results are due to the dog being present or are due to another factor plus the dog? As this is a preliminary study, I understand some things will be not included by necessity but I would encourage them to be explored in further studies. They should be discussed in the discussion too!

RE: We thank you for your comment because you gave us the opportunity to reflect on its weak points and we have included the following sentence in discussion : “Further studies concerning socio-demographic factors and other variables should be explored to state the influence of working with the dog on changing levels of serotonin and oxytocin.” (Lines 288 to 290, pag. 9)
2.2 AAA

Line 73-78: Clarity - more detail of the play and interview activities is needed. Did the dog visit with each participant? Is so, for how long? What were the interview activities? What sort of play? As this is the essential part of your study - you need to explain in detail what was done. This is the bit that anyone wanting to replicate the study needs to know. Also - how do you know it is the AAA and not just the dog being present that helped. Did you have a condition of the dog just passively in the room and not interacting?

RE: We thank you for your comment, we have added as follows: “The initial 10 min involved reintroducing the dog to each patient, the next 20 min was structured activity with the dog, 20 min interview to each patient, and the last 10 min involved the same ending activities each time (Table 1). The dog for the entire duration of the intervention remains an active part, whether it is actively acting with the play [39-40], whether it is in the dog in rest conditions on the dog mat. Its presence in the setting is that which allows the creation of a relational thrust between operator and patient and facilitates the establishment of a relationship of trust.” (Lines 122 to 128, pag. 3). as follows:

Table 1. Operative sequence of a session of AAA with the dog for dialysis patients. (Line 131 pag. 3)

<table>
<thead>
<tr>
<th>Activities</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1 Initial greetings</td>
<td>10’</td>
</tr>
<tr>
<td>Group Play with the dog:</td>
<td></td>
</tr>
<tr>
<td>Ball retrieval;</td>
<td></td>
</tr>
<tr>
<td>Step 2 Vision pictures of dog’s calming signals,</td>
<td>20’</td>
</tr>
<tr>
<td>facial expressions of the dog etc.</td>
<td></td>
</tr>
<tr>
<td>Riddles on animals</td>
<td></td>
</tr>
<tr>
<td>Step 3 Interview: Talking with the patient about</td>
<td>20’</td>
</tr>
<tr>
<td>their hobbies, style life, etc</td>
<td></td>
</tr>
<tr>
<td>Step 4 Final greetings: Dog oral discharge; Hand</td>
<td>10’</td>
</tr>
<tr>
<td>washing</td>
<td></td>
</tr>
</tbody>
</table>

2.3 Equipe

Line 79: Not sure what this heading “Equipe” means - may be unique to the journal or a Language difference. This section seems to be describing the team of people and the dog so could be listed as: the AAA team or just included in the AAA section.

RE: The correction was carried out as suggested.

Line 80-86: Clarity - this needs more detail. What sort of dog was it? Age, training? Why was it put through the Monash Canine Personality Questionnaire?

RE: this test was a useful to highlight the dog’s relational abilities. Ley et al (2009) showed his affidability. Recently, a meta-analysis demonstrated that this measure along with behavior shows high reliability over time

What sort of tasks was this dog trained in and how was its health before starting the process?

RE: The training of the dog was intended to make it able of managing frustration with self-control and reinforcements of permits adapted to the context of the setting The coping times were measured to optimize it [58] and reach a condition in which the dog expresses behavioural pleasure signals (olfactory patrol, without freezing), no demand, regular breathing, no stress signals (licking, trembling, muscle stiffness, etc.). [59]
According to the certification N ° 317jSGQ10 (in accordance to the UNI EN ISO 9001/2015), concerning the "Clinical interventions of Healthcare Zooanthropology", the dog has been endowed with a health certification issued by the Public Veterinary Service (ASL Napoli 1) containing information on health and on behavioral adequacy (i.e. documentation of current vaccinations, controllability and temperament).

How was its welfare protected throughout the study? As the welfare of the animals used in AAA is not often considered, this is very important. The dog gets no choice in taking part so readers need to know how it was handled to protect its well-being.

RE: The animal's welfare was protected by: Primary selection based on the visit of the behaviorist veterinarian on his attitude due to his attachment style. The vet is in charge of animal health and he is the owner's the dog (48-49-50-51-52-53) and they have trained together to obtain that he represents the safe base of the dog according to the principles of secure attachment. [2-3-4] The veterinarian was trained to be able to recognize in real time the communicative signals of the dog and intervene promptly to minimize the effects of the stressful. The veterinary conductor allows the dog to leave and return even during the session (55-56)

RE: We have moved the choice of the dog paragraph into "material and methods" as suggested and we have integrated the training program.

Line 81-83: Reference 12, References 13-15

RE: Thank you for the suggestion that allows us to better explain the importance that the choice and training of the operators of the team working in the health field should have in our opinion.

RE: We have improved the section AAA Team as follows: “The work team consisted in professionals trained according to interdisciplinary principles. The Psychologist and Zootherapist Veterinarian, experts in interspecific relationship work in team with an animal in situations of human illness. This is a very complex job and requires competence and responsibility. We consider it appropriate that the reference training model is of a systemic relational approach. The psychotherapist has the competence for establishing the most appropriate approach for patients in the setting with the dog, the veterinarian, has the competence on the helping relationship, but also the ability to recognize the communicative signals of the dog in real time. The vet is in charge of animal health and zoonoses, checks the suitability of the setting and creates play with the dog, (lines 133 to 141. Pg 4)

and references 16-18 need to be discussed. What did you take from these references to assess the dog in the study? It may be appropriate to move some of this information into the introduction to help explain AAA better.

RE: Thanks for your suggestion, we have improved the introduction (Lines 50 to 56, pag. 2) as follows: “The human, like other mammals, develops a necessary bond of attachment that will condition his way of relating. Furthermore, emotional ties and attachment in relationships between people and different animal species have been studied extensively[2-3-4]. The dog is the main species involved in AAls [5]. This specie has an important competence which is the ability to read the non-verbal language of the human, and moreover has developed, more than the others species, the appeal respect to the human [6].”

2.4 Blood sampling
2.5 Blood sample analysis

Line 94-101: Style - I would combine this section with that above (2.4) as one section.

RE: as you suggested we have combined the two sections 2.4 and 2.5 in a single section: 2.5 Blood sampling and analysis.

Line 94: Clarity- Were the blood tubes centrifuged immediately after collection. What sort of tubes- Plain, tubes with clotting factors added to speed clot formation?

RE: Thanks for your suggestion, we have improved the Paragraph “Blood sampling and Analisys “...in tubes for serum preparation, which were centrifuged at 1000 g at room temperature for 15 minutes, 15-30 minutes after collection. Serum was obtained and deep frozen (at −80°C) until processed as a batch for determination of serotonin and oxytocin levels.

Line 95: Style- change “...until processed for determination.” to something like “...until processed as a batch for serotonin and oxytocin levels.”

RE: The correction was carried out as suggested.

Line 95-101: Clarity- more detail is needed for the ELISA test. For example “The serum was incubated with the antigen for xx minutes at xx degrees......” This level of detail is needed to allow readers to evaluate the study data collection processes.

RE: Thanks for your suggestion, we have improved the Paragraph “Blood sampling and Analisys” as follows: “The ELISA diagnostic kits used for serotonin and oxytocin are based on a competitive procedure (Enzolife Diagnostics). Assays have been performed according to the manufacturer’s indications. Briefly, patient serum is added to a microwell plate and incubated with serotonin or oxytocin conjugated with alkaline phosphatase for 2 h at room temperature. Then anti-serotonin or anti-oxytocin antibodies are added for further incubation (for serotonin 2h at room temperature; for oxytocin 18h at 4C), followed by washing to remove the unbound antigen. Next pNpp is added for 1h at room temperature as a substrate for the alkaline phosphatase and generates a colorimetric reaction which is read in a photometer at the wavelength of 405 nm, after the reaction has been stopped” (Lines 192 to 200. Pg 5)

2.6 Statistical Analysis

Line 102-106: Good section, well reported. 3. Results

RE: Thank you for your approval.

3.1 Choice of dog.
Line 108-115: Clarity- this whole section needs to go under the materials and method section. You
didn’t have a choice of dogs- or did you? The paper reads as if there was 1 dog. What do you mean
about a good score on extraversion etc?
RE: We have moved the choice of the dog paragraph into "material and methods". as he
suggested. We reported EXTRAVERSION according to Ley et al 2009-2009b.

The conceptualisation extraversion describes the perceived energy level of the dog; self-
assuredness/motivation characterises a dog's perceived persistence in the face of distractions
(for example begging for food, finding a particular toy); training focus describes the perceived
trainability of the dog; amicability refers to how the dog is perceived to tolerate other
individuals, be they humans, dogs or other animals; and the last factor, neuroticism, describes
how cautiously or nervously a dog is perceived to behave
As yet the MCPQ (now MCPQ- R) has not been normed to give clear indication of the associations
of behaviour with different scores. If you have used information from other studies, this needs to
be indicated.
RE: This test was a useful to highlight the dog's relational abilities .Ley et al (2009) showed his
affidability. Recently, a meta-analysis demonstrated that this measure along with behavior
shows high reliability over time

Line 110-113: Clarity- what education did the owner and dog receive. Was it part of a program (a
therapy dog program) or was it some other process?
RE: thank you for your suggestion. We have integrated the training program in the section
“Choise of the dog” as follows: “The training program followed the guidelines of the national
center for sports education (CSEN Italia). All the exercises were performed to teach the
veternarian to recognize the dog’s communicative behavioural signals. The training of the dog
was intended to make it able of managing frustration with self-control and reinforcements of
permits adapted to the context of the setting The coping times were measured to optimize it
and reach a condition in which the dog expresses behavioural pleasure signals (olfactory
patrol, (without freezing), no demand, regular breathing, no stress signals (licking, trembling,
muscle stiffness, etc.). [59]All the necessary procedures have been undertaken to guarantee a
high standard of animal welfare” (lines 164 to 171. Pg 4)

How do you define a harmonious and balanced relationship? This is a scientific paper and the
writing, language and detail of what you did and how you assessed needs to be given. I have no
idea about this dog or what it was required to do from what has been written. If I want to
replicate this study or build on your findings with a similar study, I don’t know what you did or
how you did it. As this is the basic tenet of research and publishing, the lack of detail is a major
flaw in this paper.

“An harmonic interspecific relationship, the one in which the human referent represents the
safe base of the dog according to the principles of secure attachment, [2-3-4] and in which the
dog does not respond to the need to replace the human being. It is a relationship in which
reciprocity is realized. The "reciprocity" is what happens between two people, two things, two
groups so that an action or a thing received from one of the two terms corresponds to an action
or an equivalent thing for the other term. [54]. It is therefore a very complex system of
relational feedback that start from bodily gestures and attitudes, with the activation of
emotional sense-motor models between the two species. This type of the relational dimension
gives the dog an important security to be able to express itself, neutralize the stress of the first
meetings until the next adaptation [3-49-55].” (Lines 153 to 162.Pg 4)
Line 120-121: Clarity- How did you determine no behavioural and infectious problems occurred? What tests or measures did you undertake to determine this? This detail is needed.
RE: We have added to the section “Choice of the dog” a subsection titled “Behavioral and infective safety of the dog” with following paragraph:
“At T1 and T2 of each AAA session, disinfectant wipes (chlorhexidine, TRIS-EDTA, zinc gluconate and glycerin) were used for cleaning the coat, the fingertips and the tail of the dog, avoiding the transmission of zoonotic agents (e.g. bacteria, fungi, parasitic elements) [53-54-55-56]. When the dog fidgeted (for example, screaming, slapping) and did not respond to attempts to redirect its behavior, the veterinarian intervenes promptly to minimize the effects of the stressful situation by changing the activity, maintaining a distance between the dog and the stressful stimulus, or put the dog in resting condition on the mat with an "oral discharge." (lines 173 to 180 Pg 4)

3.2 Behaviour of the dog
Line 116-121: Clarity- this section is of no value to the paper. It is not clear what you are trying to say or how you assessed motivation, joy and adaptation.
RE: We apologize for the lack of clarity. Following your suggestions, we considered this session useless and removed it.

3.3 Statistical Analysis
Line 129: “Per each phase...” should be “For each phase...” Line 132-137: There should be p-values stated here.
RE: We apologise for lack of information. We added p-values within the text: the comparison between models was significant (p<0.05).

Line 138: Clarity- last sentence should read “In figure 3 the common biphasic pattern of serotonin and oxytocin serum levels are shown.”
RE: The sentence was rephrased accordingly.

3.4 Figures
Figure 1
Line 141: T1 and T2 are referred to but in your description of the process of the study and the data analysis, you do not refer to T2- to what part of the research does it refer? Check for this type of inconsistency.
RE: We apologize for the error, we have changed in the text therefore T0 is now T1 and T1 is now T2.

Figure 2
Line 145: You need to label the two parts of the figure with (a) and (b). Also T2 has been referred to again. Do you mean T0 and T1?
RE: we have changed in the text therefore T0 is now T1 and T1 is now T2.

Figure 3
Line 152: Clarity- is there meant to be a (b) figure for Figure 3? If not there is an error in the caption for figure 3.
RE: We apologize for the error we removed (b) from the caption
4. Discussion

Comments: The discussion lacks depth and detail in its assessment of the results. What did you find—that the serotonin and oxytocin varied together. Why might this be?

: “The interaction between oxytocin and serotonin, in studies of animal models, it is described that social interaction requires the coordinated activity of Oxitocin and Serotonin in the nucleus accumbens, similar as in humans.”

You need the detail of the AAA and the human patients to know if the AAA was the reason for the variation of the serotonin and oxytocin. How does the serotonin nd oxytocin variance affect the patients mood. This is not clear. What is happening over the first 4 weeks when the serotonin and oxytocin drop- are the patients depressed or happy? And then the remaining 7 weeks what happens to them?

RE: We have integrated the Discussion like follows.

From these preliminary results we observed that the AAA with the dog can induce a release of serotonin and oxytocin... However, this improvement, remains only for the time of work because in the checks that carried out in the following months, serotonin and oxytocin levels settled approximately on the initial values. The results display an interesting trend also evident on the behaviour of the patients. who had been chosen through the behavioral observations of the psychologist who reported the an important tendency to isolate themselves and to remain silent, with little possibility of dialogue between them or with the medical staff... (Lines 266 to 270. Pg 8)

Line 159-160: This sentence does not make sense. You did not describe the behaviour of the patients. This would be good to know because.

RE: The sentence has been inserted in the text as follows: “...They, in fact, increased sociality in the group and the demand for participation in the plays with dog. They were became more colloquial among themselves and with the veterinarian and more feel care with the dog (someone brought from home prizes for dogs) In the interview they reported as pleasure of the hour of therapy in which the dog was in respect to the other hours, as an improvement in mood when they come back home in the day of AAA”. (Lines 267 to 272. Pg 8)

Line 163: Clarity- check and use either serotonin or 5-HT throughout the paper. I would suggest serotonin is better as it is clearer (same goes for OXT or Oxytocin).

RE: The correction was carried out as suggested.

Line 165: Clarity- there is unnecessary repetition of the “OXT receptors are expressed on serotonergic cells”. This needs to be edited. Assume the reader is not completely familiar with the effect of oxytocin on the serotonergic cells- inhibitory I assume from your writing, but it is not clear.

RE: We apologize for the error, the sentence was removed as suggested.

Line 168: When referring to other work by the same group of authors/research group, I would recommend mentioning that fact.

RE: The correction was carried out as suggested.

5 Conclusions:

Line 172-173: This sentence doesn’t make sense. The relational competence of the dog and the relationship with the vets who was also the owner were not defined and the importance of this to the outcomes of the study have not been explained in this paper.
RE: We thank you for the suggestion and reformulate the sentence as follows in the paragraph “choise of the dog”: “We gave importance to the relational competence of the dog and to the relationship with the veterinarian who was also the owner. From the consulted literature it emerges that the attachment style influences the relationship mode both in the man and in the dog and the dog makes a difference between the owner and other people, improves his performances. Even the studies of Pratoprevide and other authors say that the dog in the presence of its owner shows greater security towards new stimuli.”(line 148 to 153 pg 4) These conditions are also important for total safety from possible zoonotic risks (line 163 pg 4)

Line 176: As you do not include any measures of patient well-being beyond a hypothesized relationship between patient well-being and serotonin and oxytocin levels (with no discussion of the nature of the relationship), this statement in your conclusion is invalid. You cannot hypothesize in a conclusion - you are answering your hypothesis in the conclusion. Do your results support your hypothesis or not?

RE: Thank you for your suggestion. The paragraph was modified like follows: “From these preliminary results, we observed that the AAAs with the dog induce a release of serotonin and oxytocin after an initial decrease, that we hypothesized as due to the novelty and the necessary adaptation. A significant increase of serotonin and oxytocin was then observed in the subsequent sessions. However, no significant difference was detected between the beginning and the end of the same session, possibly due to individual variable levels. Finally, the increase of serotonin and oxytocin was not detected in the determinations carried out after the termination of the activities, as serotonin and oxytocin levels settled approximatively on the initial values. Further studies are necessary to deeply evaluate the results obtained” (lines 294 to 302. Pg 9)

Line 177: Conclusions do not generally have references. This new paper should be in the discussion if you need to refer to it.
RE: As you suggested we have removed it.

Line 178-180: The weak points of the study need to be considered in the Discussion along with areas for future studies.
RE: Thank you for your suggestion, we moved the weak point in the Discussion as suggested.

References:
Check all references - there are errors in author lists (line 234: this paper has 3 authors but has 4 listed, 2 by their first names not surnames)
RE: As you suggested we have corrected it.