

Does attachment to man already exist in 2 months old normally raised dog puppies? A pilot study

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Abstract: Based on the current knowledge, adult dogs are able to establish an attachment bond towards their owners and puppies are able to establish an attachment bond towards their mother. The aim of the current study was to assess whether dog attachment to people already exists in puppies.

Fourteen 2 months old puppies were tested in the Ainsworth Strange Situation test in which the role of the human caregiver was played by the person with whom puppies had interacted more in their first weeks of life, i.e. their breeder.

As expected, puppies displayed more protest at separation (whining, behaviors against the door, close to the door etc.) when involuntarily separated from the breeder. They also increased the seeking of proximity after separation, but they did not show a clear preference for the breeder over a human stranger. The duration of exploration, play and resting seem to be related to the order of episodes more than to a secure base effect.

The findings of this preliminary study do not fully support the hypothesis that dogs establish an attachment bond towards their human caregivers since puppies, provided that they are raised by their mother. It is likely that a longer time spent together is needed for this peculiar kind of bond to be formed.

Key Words: Ainsworth Strange Situation Test, attachment, breeder, dog, puppy, secure base.

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Introduction

All modern dog breeds derive from an ancestor who was domesticated over 15,000 years ago (Grimm, 2015), and subsequently dogs were subjected to an intense process of artificial selection. This process has been suggested to promote general socialization (Kretchmer & Fox, 1975) and predispose dogs to form attachment towards humans (Topál et al., 2005).

Attachment can be defined as an affectional tie that an individual forms to another specific one, binding them together in space and enduring over time (Ainsworth & Bell, 1970). The theory of attachment currently known has been developed by Bowlby (1969), one of the first authors who tried to pool the results of research on the bond between young animals and their mothers, regardless of the species they belonged to. The behavioral test commonly used to study child attachment to the mother is called Ainsworth's strange situation test (ASST; Ainsworth & Bell, 1970). Modified versions of the same test have also been used for testing the young of other mammalian species towards their mother (e.g. Bard, 1991) or to individuals other than the mother (e.g. Bard & Nadler, 1983 in young chimpanzees towards their peers), including subjects belonging to a different species (e.g. chimpanzees towards a human caregiver: Miller et al., 1990, 1986). Although the presence of an attachment bond is particularly important in young animals, being protection its main function, attachment persists into adulthood (e.g. McConnell & Moss, 2011).

Topál et al. (1998), hypothesizing a similarity between dog-owner and child-mother relationship, adapted the ASST to make it suitable to test adult dogs with their caregivers, i.e. the owners. Since then, many studies have been carried out to study dog bond towards humans (for a review see Payne et al., 2016). Only recently research has also focused on dog-dog bond and used ASST to analyze canine intraspecific attachment. Previde et al. (2009) found that dog puppies tested in the ASST showed patterns of behaviors indicative of an attachment bond towards their mothers, as expected when testing young mammals with their primary caregivers. Two studies carried out on adult dogs living in the same household (Mariti et al., 2014; Mariti et al., 2017) instead suggest that the relationship between two adult dogs having shared the same environment for a long time does not fulfill all the requirements needed to properly call this relationship an attachment bond. However, the findings of Mariti et al. (2017) suggest that the puppy-mother bond can be somewhat maintained, provided the possibility to keep living together.

Based on the current knowledge, i.e. adult dogs are able to establish an attachment bond towards their owners (Mariti et al., 2013) and puppies are able to establish an attachment bond towards their mothers (Previde et al., 2009), it can be hypothesized that puppies establish an attachment bond towards their owners. Consequently, the aim of the current study was to assess whether dog attachment to people already exists in puppies. As most puppies are raised by their mother, only normally raised (not hand raised) puppies were included in this pilot study.

Subjects, materials and methods

Participants

Fourteen puppies were involved in this study. All puppies were 2 months old (58-60 days of age), equally divided per sex, belonging to five litters of different breeds (2 Australian Kelpies, 3 Bearded Collies, 3 Border Collies, 3 Flat Coated Retrievers, and 3 Labrador Retrievers). All puppies were raised in a home environment and still lived in the household where they were born and raised by their mother, i.e. puppies were tested before being adopted by a new family.

The role of the human caregiver in the ASST was played by the person with whom puppies had interacted more in their first weeks of life, i.e. their breeder. For the current study, only female 25-35 years old breeders were included, in order to match their characteristics with those of the stranger as far as possible. Breeders were all volunteers recruited by personal contacts among people who could guarantee that puppies had regular and positive contact with them.

Experimental setting

The experimental environment was a relatively bare room, 4.50 x 4.30 m, unfamiliar to the puppies. The room was prepared to match as far as possible the requirements described in the ASST (Ainsworth & Bell, 1970), especially in the version modified for dogs. The room was equipped with two chairs, one for the breeder and one for the stranger; a water bowl; three dog toys (a Kong*, a little ball, and a knotted rope); a table to leave the leash on; and two video cameras to record the whole test. One video camera was oriented to the door and the surrounding area, whereas the other one recorded the whole room.

To avoid external noise, tests were conducted during the weekend, when the facility was not in use. At the end of each test, the experimental room's floor was washed using a nontoxic weakly scented disinfectant.

Procedure

The procedure was as faithful as possible to the Ainsworth Strange Situation Test (Ainsworth & Bell, 1970). Seven 2-min episodes were carried out in an experimental room, unfamiliar to all puppies. As recommended in the ASST, the procedure included two separations from the presumed attachment figure, i.e. the breeder (episode 3, puppy with stranger; episode 5, puppy in complete isolation; and episode 6, puppy with stranger) and two reunions with her (episode 4 and 7). A more detailed description of the procedure is reported in Table 1.

Table 1. Behavioral test used for this study. Each episode lasted 2 minutes.

Episodes	Subjects involved	Description		
1	Puppy + Owner	Owner and puppy entered the experimental room. The puppy was unleashed and set free to explore the room. The leash was placed on the table. Owner sat on the chair and interact with the puppy only if he/she performed attention seeking behaviors.		
2	Puppy + Owner + Stranger	The stranger entered the experimental room and sat in the free chair. Participants couldn't talk to each other and interact with the puppy only if he/ she performed attention seeking behaviors. At the end of the second minute, the owner left the room.		
3	Puppy + Stranger	The owner was out of the room (taken in another room 20 m away from the experimental room), the puppy was with the stranger in the room.		
4	Puppy + Owner	The owner came back into the room and sat into the same chair. In the meanwhile, the stranger left the room. If the puppy initiated interaction, the owner greeted and comforted the animal as he/she usually does at reunion. At the end of the second minute, the owner left the room.		
5	Puppy	The puppy was left alone in the room.		
6	Puppy + Stranger	The stranger came back into the room and sat on the same chair. If the puppy initiated interaction, the stranger greeted and comforted the puppy.		
7	Puppy + Owner	The owner came back into the room and sat into the same chair. If the puppy initiated interaction, the owner greeted and comforted the animal as he/she usually does at reunion. In the meanwhile, the stranger left the room.		

The owners and the stranger were asked to not interact with the puppy, and to remain seated during the whole procedure, except when they had to leave or come back into the room.

Puppies were videotaped throughout the test and their behavior was analyzed using a continuous sampling method in order to measure the duration (in seconds) of puppies' social and non-social behaviors; social behaviors were analyzed both towards the stranger and towards the breeder. The list of analyzed behaviors is reported in table 2 and 3; they correspond to behaviors commonly used for the analysis of dog behavior in the ASST (see e.g. Mariti et al., 2013; Topál et al., 1998).

Statistical analysis

The statistical analysis consisted in comparing the duration of each behavior for all the episodes using a Wilcoxon paired-sample test (multiple comparison corrections were performed using the Benjamini-Hochberg procedure) run with the software SPSS Statistic 17.0 (Chicago, IL).

Results

Comparisons resulting in statistically significant differences are summarised in Tables 2 and 3. Behaviors non reported in the tables did not result significantly different in any of the performed analyses.

Exploration was found to be longer in episode 1 compared to all the other episodes; on the contrary, resting was shorter in episode 1 than in any other episode.

Proximity was higher in later episodes, after separation. Visual orientation to people showed a different trend.

Whining and behaviors against the door were displayed statistically more in episode 5 (complete isolation); locomotion, staying close to the door and being visually oriented at the door were longer in episode 5 and 3 (first separation from the breeder).

Table 2. Summary of statistically significant results for proximity seeking and secure base effect. For episode 2, being owner and stranger present at the same time, o=owner and s=stranger.

	Behaviors	Episodes	Statistical values
Secure base	Exploration	1 > 2	W = -2.975, P = 0.003
		1 > 3	W = -2.668, P = 0.008
		1 > 4	W = -3.061, P = 0.002
		1 > 5	W = -2.481, P = 0.013
		1 > 6	W = -3.076, P = 0.002
		1 > 7	W = -3.041, P = 0.002
		2 > 6	W = -2.197, P = 0.028
	Individual play	1 > 3	W = -2.675, P = 0.007
	Resting	1 < 2	W = -1.992, P = 0.046
		1 < 3	W = -1.992, P = 0.046
		1 < 4	W = -2.028, P = 0.043
		1 < 5	W = -2.197, P = 0.028
		1 < 6	W = 2.197, P = 0.028
		1 < 7	W = -2.666, P = 0.008
Proximity seeking	Attention seeking	6 > 4	W = -2.214, P = 0.027
	Social exploration of owner	2(o) < 4	W = -2.040, P = 0.041
		2(o) < 7	W = 2.240, P = 0.025
	Proximity to owner	7 > 1	W = -2.062, P = 0.039
		7 > 2(o)	W = -2.276, P = 0.023
	Proximity to stranger	6 > 2(s)	W = -2.552, P = 0.011
	Proximity owner versus stranger	7 > 2(s)	W = -2.272, P = 0.023
		7 > 3	W = -2.063, P = 0.039
	Social exploration of owner versus stranger	2(s) > 2(o)	W = -2.298, P = 0.022
		6 > 2(o)	W = -2.100, P = 0.036
	Visual orientation to owner	1 > 2(o)	W = -2,590, P = 0.010
		4 > 2(o)	W = -3.070, P = 0.002
		7 > 2(o)	W = -2.909, P = 0004
	Visual orientation to owner versus stranger	2(s) > 2(o)	W = -2.654, P = 0.008
		3 > 2(o)	W = -2.812, P = 0.005
		6 > 2(o)	W = -2.852, P = 0.002

Table 3. Summary of statistically significant results for protest at separation.

Behaviors	Episodes	Statistical values
Whining	5 > 1	W = -2.448, P = 0.014
	5 > 2	W = -2.599, P = 0.009
	5 > 3	W = -2.666, P = 0.008
	5 > 4	W = -2.192, P = 0.028
	5 > 6	W = -2.666, P = 0.008
	5 > 7	W = -2.666, P = 0.008
Locomotion	5 > 2	W = -2,184, P = 0.029
	5 > 3	W = -2.199, P = 0.028
	5 > 6	W = -2.521, P = 0.012
	5 > 7	W = -2.242, P = 0.025
	3 > 6	W = -2.383, P = 0.017
Visual orientation to door	3 > 1	W = -2.207, P = 0.027
	3 > 2	W = -2.269, P = 0.023
	3 > 4	W = -2.674, P = 0.007
	3 > 6	W = -2.075, P = 0.038
	3 > 7	W = -2.458, P = 0.014
	5 > 2	W = -2.941, P = 0.003
	5 > 4	W = -2.075, P = 0.038
	5 > 6	W = -2.670, P = 0.008
	5 > 7	W = -2.675, P = 0.007
Close to door	5 > 4	W = -2.197, P = 0.028
	5 > 6	W = -2.371, P = 0.018
	3 > 4	W = -2.214, P = 0.027
	3 > 7	W = -2.201, P = 0.028
Behaviors against door	5 > 1	W = -1.963, P = 0.050
-	5 > 2	W = -1.997, P = 0.046
	5 > 3	W = -2.201, P = 0.028
	5 > 4	W = -1.997, P = 0.046
	5 > 6	W = -1.997, P = 0.046
	5 > 7	W = -2.207, P = 0.027

Discussion

In order to test the hypothesis that dogs form an attachment bond to people since puppies, we tested 2-months old puppies in the ASST with their human caregiver, i.e. their breeder, and we assessed if that relationship met the three main criteria of an attachment bond: protest at an involuntary separation from the attachment figure; seeking proximity with the attachment figure (especially upon reunion); and secure base effect.

Data reported in table 3 shows that puppies displayed behaviors indicative of protest at separation during episode 3 and 5, in which puppies were expected to show it because in these two episodes puppies were involuntarily separated from the presumed attachment figure, i.e. the breeder. In addition, dog puppies in this study showed more behaviors indicative of stress in a later episode, when completely alone (episode 5), than in a previous episode in which a stranger was present in the experimental room (episode 3). This is also an expected finding: in fact, the ASST is sometimes described as a "drama in miniature" in which the young animal is subjected to a mild and increasing stressful situation. It must be taken into account that, contrary to adult dogs,

puppies usually are not used to stay alone (or not for a long time), as the mother tends to spend much time with the litter, and littermates usually stay together. It is therefore likely that puppies show high distress at separation when completely alone, especially if the mother has provided a high amount of maternal care (Guardini et al., 2015; 2017). In addition, puppies are less used than adult dogs to visit novel environments, and these two conditions put puppies in a situation very similar to that of children tested in ASST (more similar than that of adult dogs). Finally, puppies here were tested when separated from the breeder in a condition that adds to the separation from the mother (never present during the test), thus creating a sort of double separation, as hypothesized for dogs tested with conspecifics in the ASST (Mariti et al., 2018). In view of the above, the findings on protest at separation are compatible with the presence of an attachment bond between puppies and their breeder.

As for the second requirement of an attachment bond, when analyzing the seeking proximity effect in the ASST, two results are expected: the tested subject prefers the proximity of the caregiver over a stranger; and proximity seeking increases after separation, due to the activation of the attachment behavioral system. The latter point was confirmed by an increase in proximity upon reunion with both the stranger (episode 6 > 2) and the breeder (episode 7 > 1 and 2). Instead, proximity to the caregiver was only partially found to be more displayed than proximity to the stranger (episode 7 > 2 and 3, but no difference between episode 6 and 7 nor between 3 and 4).

The lack of difference between the early episodes with the owner (1, 2 and 4) and early episodes with the stranger (2 and 3) seem to suggest that puppies did not prefer the breeder over a stranger. However, such findings must be discussed cautiously, considering the peculiarities of dog puppies compared to children tested in ASST.

As stressed by Rehn & Keeling (2016), literature on dog relationship with people is sometimes unclear about the difference between attachment behavior and attachment bond. Ainsworth & Bell (1970) defined attachment behavior as those behaviors aimed at promoting and restoring proximity and contact. Bowlby (1988) stated that almost all children, when distressed, go to the mother; in case she is absent, children fall back on somebody else. In such conditions the majority of children show a clear hierarchy of preferences and, in case nobody else is available, even a gentle stranger can be approached. So the Author stressed that, while the attachment behavior can be displayed in different conditions towards many subjects, a lasting attachment, or attachment bond, is reserved to very few people (Bowlby, 1988). However, it must be taken into account that dogs and children usually show a very different response to a stranger. Being Canis familiaris a highly social species (Tuber et al., 1996), domestic dogs naturally seek social interaction, unless they lack for socialization or had very negative experiences. Therefore, contrary to children tested in the ASST, at 2 months of age well-socialized puppies usually do not show fear of stranger. In particular, dog puppies at 8 weeks of age, as those we tested, show less fear than younger ones (Marshall-Pescini et al., 2017). Indeed, the findings of episode 2 (dog-owner-stranger) for seeking attention towards the stranger, social exploration and visual orientation suggest that puppies were not wary of the stranger, rather they sought more attention from the stranger than from the breeder. In other terms, in the current study puppies behave similarly to adult dogs, and differently from children (Mariti et al., 2013; Mariti et al., 2017), interacting with the stranger since her entrance. Social exploration and visual orientation suggest curiosity towards the stranger, compatible with neophilia (Kaulfuss & Mills, 2008), and it can hinder the expected difference in proximity seeking with the breeder over a stranger.

Similarly to what found for proximity seeking in early episodes, the lack of difference after a complete isolation, upon the second reunion with the stranger (episode 6) and with the breeder (episode 7), does not confirm a preference for the caregiver over a stranger. However, these findings need a deeper discussion that also takes into account the findings of attention seeking. Puppies spent statistically more time in attention seeking from the stranger after complete isolation compared to upon the first reunion with the breeder. Such result confirms that the presence

of a human, although stranger, has a strong ameliorative effect, as already found in young and adult dogs (Mariti et al., 2017, 2013; Palmer & Custance, 2008; Tuber et al., 1996; Pettijohn et al., 1977). However, adult dogs show a clear preference for the owner over a stranger (Mariti et al., 2013; Topál et al., 1998). Instead, the findings of the current study seem to suggest that dog puppies, when feeling distressed, do not clearly differentiate between a familiar and an unfamiliar human as a source for alleviation of distress. Further studies are needed to clarify if the relief obtained through the reunion with the stranger is sufficient to reestablish the homeostasis in the activated attachment behavioral system or if the presence of the breeder (or of the mother) is needed.

Generally speaking, many aspects of puppies' bond and social preferences need to be better investigated. According to Ainsworth & Bell (1970), although the presence of another individual with whom the human infant has an affectional bond (i.e. a same age play mate) may provide some comfort, the child will not usually be fully comforted or secure until she or he is reunited with the attachment figure. Previous studies on dog puppies do not show a clear picture. For instance, 8 weeks old puppies display signs indicative of an attachment bond towards the mother (Previde et al., 2009) but do not show a preference for the mother over littermates after a 10-sec isolation (May et al., 2009). Nor puppies prefer the mother over a human stranger for the alleviation of separation distress (Pettijohn et al., 1977). Once grown up, dogs prefer an unfamiliar person over an older female cohabitant dog, but behave similarly towards the mother and towards a human stranger (Mariti et al., 2017), underlying how difficult and complex is comparing dog-dog and dog-human relationships (Mariti et al., 2014).

The secure base effect is, according to Ainsworth, the feature that mainly distinguishes an attachment bond from other affectional ties. Owners can represent a secure base for their adult dogs in the ASST (Mariti et al., 2013) as well as in other situations (Gácsi et al., 2013; Horn et al., 2013), and recent studies suggest that dog-owner bond can present the all the styles found in child-mother attachment (Schöberl et al., 2016). The secure base in dog-human relationship is investigated through the analysis of exploration and play behavior, which should be more displayed in the presence of the attachment figure (Mariti et al., 2013). In the current study, as expected from previous studies in adult dogs (Prato-Previde et al., 2003), puppies showed higher levels of exploration when the environment was truly novel, i.e. in the first episode of ASST, and then decreased. The longer exploration in episode 2 compared to episode 6 can probably be included in the trend of reduction through the procedure, more than been explained by the fact that in episode 2 the puppy and the stranger were in the company of the owner.

In case the secure base effect is present, puppies should have felt safer when together with the owner, and therefore more prone to rest in the episodes with her. Although lower levels of resting were shown in episode 1, i.e. when the owner was in the room, this result may be the other side of the coin regarding explorative behavior: in the first episode puppies were curious of the new environment, so they did not rest. The increase of resting in the last episode may also be indicative of tiredness, that is more likely to occur in young animals than in adults.

In the current study, individual play was displayed for a statistically longer time in episode 1 than in episode 3. As for exploration, it is possible that such finding is not related to the presence of the owner bur rather to the order of episodes within the test. The results obtained for exploration, individual play and resting suggest that puppies were more active at the very beginning of the test and then their interest towards the environment was reduced, as occurs for adult dogs (Prato-Previde et al., 2003). Taken together, these results do not clearly indicate a secure base effect of the breeder for the puppy.

Considering the lack of evidence of a secure base effect, the findings of the current study do not fully support the hypothesis that 2-months old, normally raised dog puppies establish an attachment bond towards their human caregiver. This is partially in disagreement with previous literature, showing that the attachment system varies with age in domestic dogs, and in detail

young dogs tend to show more evident attachment behaviors (Carlone et al., 2014), whilst aged dogs show more evident physiological changes but less behavioral indicators after the activation of the attachment system (Mongillo et al., 2013).

The findings of this study should be regarded as preliminary, due to the novelty of the study and the relatively small sample. Anyway, they represent a starting point from which to explore the development of dog-man relationship.

Our findings are apparently in disagreement with those of Topàl et al., (2005), who found that 4 months old dog puppies (hand-raised and normally raised) behave differently from wolf cubs in the ASST, showing attachment behaviors towards the owner. However, the behavior of puppies at 8 and 16 weeks of age can be quite different, as well as the kind of relationship established with other individuals. In addition, a direct comparison of our results with that study cannot be done, as Topál et al. (2005) did not analyze single episodes and the human caregiver of pet puppies was not the breeder but the adopter (personal communication). The importance of owners' behavior in creating a relationship with a dog (Schöberl et al., 2016), including the shared activities and the time spent together (Carlone et al., in press), can be responsible for the discrepancy between conclusions drawn by Topál et al., (2005) with 4 months puppies, and our conclusions on 2 months old puppies.

Another possible explanation for our results is that 2 months old puppies have established a primary attachment bond towards the mother, still ongoing, and that bond may hinder the establishment of other bonds, e.g. towards the human caregiver. Further studies are needed to better understand this point. All young animals, including puppies, need to establish an attachment bond; so, if a person represents the primary caregiver, this person is likely to become the attachment figure. It is possible that a study focusing on hand-raised, 2 months old puppies may have led to different results. However, this pilot study was focused on puppies raised by their mother, as they represent the majority of puppies.

A possible practical implication of these preliminary findings is related to the age of adoption. If puppies are adopted by a new family when they are 2 months, probably they have to face a disruption from the mother and littermates but not from the breeder; when adopted at a later age, puppies may have to undergo also a disruption of the relationship with a human, which may cause higher stress in the short term and more difficulty in creating a secure bond with the new owner in the long term (Prato-Previde et al., 2007).

Conclusions

The findings of this preliminary study do not fully support the hypothesis that dogs establish an attachment bond towards their human caregivers since puppies, provided that they are raised by their mother. It is likely that a longer time spent together is needed for this peculiar kind of bond to be formed and/or that the primary attachment bond towards the mother, still present in puppies, hinders the establishment of other bonds.

Further studies should focus on the relationship between puppies and adopting owners, in order to assess the time needed for establishing a bond that can be properly called attachment and which factors can facilitate its formation.

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Ethical considerations

All procedures were performed in full accordance with the Directive 2010/63/EU of the European Parliament and of the Council of 22/09/2010 on the protection of animals used for scientific purposes and conformed to the "Guidelines for the treatment of animals in behavioral research and teaching" (Behaviour, 2016). As this study is observational in nature, it did not require an approval from an ethical committee.

Before starting the procedure, the participating owners were introduced to the study, their role, and what to expect by participating. They were then asked to complete a consent form authorizing the use of collected data in accordance with the Italian National Privacy Law valid at the time the study was carried out (Legislative Decree n. 196, June 30, 2003).

Before the tests owners were told that the purpose of the study was to analyze dog explorative behavior; the complete aim was disclosed after the participation at the test, in order to avoid that owners would modify their behaviour according to it.

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L'attaccamento all'essere umano esiste già in cuccioli di due mesi allevati dalla madre? Uno studio pilota

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Sintesi

In base alle attuali conoscenze, i cani adulti sono in grado di stabilire un legame di attaccamento verso i proprietari e i cuccioli nei confronti delle proprie madri.

Lo scopo di questa ricerca è stato quello di verificare se nei cuccioli esiste già un attaccamento nei confronti delle persone.

Sono stati testati 14 cuccioli di due mesi di età, nel test di Ainsworth della Strange Situation, in cui il ruolo della persona che si era presa cura dei cuccioli era svolto dall'allevatore, essendo l'essere umano con cui i cuccioli avevano interagito maggiormente nelle prime loro settimane di vita.

Come previsto, i cuccioli protestavano alla separazione dall'allevatore, uggiolando, esibendo comportamenti orientati verso la porta e rimanendo nei pressi della porta.

Inoltre i cuccioli aumentavano la ricerca di prossimità dopo la separazione ma non mostravano una chiara preferenza per l'allevatore, rispetto ad una persona estranea.

La durata dell'esplorazione, del gioco e del rimanere fermo sembrano essere correlati all'ordine degli episodi piuttosto che all'effetto base sicura.

I risultati di questo studio preliminare non confermano completamente l'ipotesi che i cani, da cuccioli, stabiliscano nei confronti della persona che si prende cura di loro, se è presente la madre. È verosimile che sia necessario che gli animali trascorrano con la persona un periodo di tempo più perché questa particolare forma di legame si formi.