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Title: Association between exposure to farm animals and pets and risk of
Multiple Sclerosis

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Keywords: Multiple Sclerosis, pets, farm animals, remoteness, cat, dog,
case-control study, risk factor

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Terence Dwyer; Ingrid van der Mei

Abstract: Purpose: There exists inconsistent evidence regarding animals including pets as risk factors for the development of multiple sclerosis (MS). We investigated the association between farm animals and pets as possible environmental factors in MS development.

Methods: Population based case-control study with 136 clinically definite MS cases and 272 controls randomly chosen from the community matched on sex and age. Data was collected from both questionnaire and a lifetime calendar detailing residence, occupation and pet/animal exposure over the course of participant's lives.

Results: Exposure to farming, livestock, specific farm animals and remoteness of residence showed no significant association with MS risk. Exposure to cats prior to disease onset was associated with a greater risk of MS (Adjusted Odds Ratio 2.46 (1.17-5.18)) but without a clear dose-response (test for trend, $p=0.76$).

Conclusions: In contrast to other literature, farming and exposure to farm animals were not associated with MS. While we identified an association between cat exposure and MS, there was no dose-response relationship, and previous studies showed inconsistent results, leaving us to conclude that there is no strong evidence that exposure to cats is associated with MS.

Reply to reviewers' comments – resubmission 2

Reviewer #1: While the paper is much improved with the additional information about study design and methods, I tend to agree with the other reviewer in that the actual information adds very little. I maybe would change the focus of the additional conclusions to suggest that exposure to other animals including livestock and occupation were not associated with MS. This is perhaps more important information rather than the information on the potential cat exposure association (given the number of hypotheses tested, it's likely this is a spurious association - especially since cat exposure specifically was not the primary aim of the study).

We are fine with this suggestion. We have changed the order of our findings and are now discussing the associations with other animals including livestock and occupation first, and then the findings about cats. This places more emphasis on our primary hypothesis and de-emphasises the association with cats.

We have done this in the following sections:

- Abstract, results
- Abstract, conclusion
- Results – table 2
- Results - text
- Discussion, first paragraph where we summarised the results

The number of hypotheses tested (of different types of animals/occupations) should be mentioned as a limitation.

We have included this now as a limitation in the discussion and stated: “We tested a substantial number of pet and farming-related variables which increased the chance of identifying a false-positive association.”

"While we identified an association between cat exposure and MS, there was no dose-response relationship, and previous studies showed inconsistent results, leaving us to conclude that there is no strong evidence that exposure to cats is associated with MS." This phrase appears 2 times in the article and is the highlight - I suggest that it should be reworded such that it only appears once.

- We removed this text from the highlight.
- We removed this text from the conclusion in the discussion.

Reviewer #2: The authors have addressed the concerns and acknowledged the limitation of the research in the revised manuscript.

Highlights

- ~~While we identified an association between cat exposure and MS, there was no dose-response relationship, and previous studies showed inconsistent results, leaving us to conclude that there is no strong evidence that exposure to cats is associated with MS.~~
- In contrast to other literature, farming, ~~and~~ exposure to livestock or specific farm animals ~~waeres~~ not associated with MS.

Association between exposure to farm animals and pets and risk of Multiple Sclerosis

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Running title: Farm animal and pet exposure and MS

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factor

Abstract

Purpose: There exists inconsistent evidence regarding animals including pets as risk factors for the development of multiple sclerosis (MS). We investigated the association between farm animals and pets as possible environmental factors in MS development.

Methods: Population based case-control study with 136 clinically definite MS cases and 272 controls randomly chosen from the community matched on sex and age. Data was collected from both questionnaire and a lifetime calendar detailing residence, occupation and pet/animal exposure over the course of participant's lives.

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Conclusions: In contrast to other literature, farming and exposure to farm animals were not associated with MS. While we identified an association between cat exposure and MS, there was no dose-response relationship, and previous studies showed inconsistent results, leaving us to conclude that there is no strong evidence that exposure to cats is associated with MS.

INTRODUCTION

Multiple Sclerosis (MS) is a complex disease of the central nervous system, and aetiological factors continue to be uncovered[1]. Contagious viral illnesses of pets, such as canine distemper, may potentially be associated with an increased risk of MS. However, immune modulation as a result of exposure to pets could also have a beneficial effect, in line with the “hygiene hypothesis”, which proposes that early life infections may down-regulate allergic and autoimmune disorders[2]. Inconsistent evidence exists regarding the association between exposure to pets and/or other animals and MS risk[3-5], but our group recently identified a positive association between farming and exposure to livestock and risk of central nervous system demyelination[6]. In a population based case-control study in Tasmania, we examined whether farming, and exposure to pets and farm animals prior to the onset of MS was associated with MS risk.

METHODOLOGY

Study Participants

The population is described in greater detail elsewhere[7,8]. People with MS under the age of 60 years were recruited in the state of Tasmania through the use of advertising, information evenings and letters from neurologists. In total, 169 subjects volunteered to participate, while 136 cases were included in the final sample. Thirty (17.8%) did not meet the study protocol with regard to the diagnosis of MS, one person refused a neurological assessment, one person died before the interview took place and one person deteriorated to the extent that the disease became too severe to participate. Case respondents were interviewed and examined by one of the participating neurologists. Magnetic resonance images (MRI) were assessed for 134 of the 136 the cases, and for the other two cases MRI reports from previously conducted scans were obtained. The eligible cases had cerebral MRI abnormalities consistent with MS, as defined by Paty et al.,[9] and definite MS using the criteria of Poser et al.[10] Cases with a classification of primary progressive MS had to exhibit progressive neurological disability for at least one year, had to have no other better

1 explanation for the clinical features, and had to have relevant spinal cord abnormalities and changes
2 on cerebral MRI consistent with demyelination. Controls were selected from the roll of registered
3 electors, a comprehensive listing of the population maintained by the State Electoral Office of
4 Tasmania. For each verified case, two control subjects were randomly selected and matched to the
5 index case on sex and birth year. For the 136 cases included in the study, 272 eligible controls
6 participated with a response rate of 76%.
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13 All participants provided written consent with ethics approval being granted by the Human
14 Research Ethics Committee of the Royal Hobart Hospital.
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23 *Measures*

24 Data was collected by a partly self-completed life and lifetime calendar and a face-to-face interview
25 was conducted by one of two research assistants between March 1999 and June 2001. For each year
26 of their life participants indicated location of residence, the occupation they had, whether they lived
27 at a farm, whether they had any farm animals and whether they had any pets at home that were
28 owned by members of the household, including the type and number of pets. Regarding dog
29 exposure, during the face-to-face interview, the interviewer would ask where the dog would mostly
30 spend its time (mostly outdoors, more outdoors than indoors, more indoors than outdoors, mostly
31 outdoors), how often the participant would cuddle, pat, nurse or stroke the dog (less than 3 times a
32 day, 3-6 times a day, more than 6 times a day) and whether the dog had a disease at a certain stage
33 of life. Residential locations were classified according to the Australian Standard Geographical
34 Classification (ASGC) as a proxy for exposure to a rural environment.
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55 *Data Analysis*

56 Using the yearly data from the calendar allowed the calculation of the time (in years) spent in
57 contact with pets prior to the age of first symptom (and the same age for each matched control) or
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average remoteness index and the calculation of exposure prior particular ages, to examine whether some age periods were of particular importance. Odds ratios and 95% confidence intervals were estimated by conditional logistic regression. We examined a number of factors as potential confounders as they were associated with MS in this dataset[7,8]. Farm and pet exposures were adjusted for smoking prior to the onset (no, yes) and time spent in the sun during weekends and holidays before the age of 16 ($\geq 2-3$ vs $\leq 1-2$ hrs/day) as e.g those with a cat were less likely to be smokers and more likely to be exposed to the sun. Remoteness variables were adjusted for education level and whether participants had exposure to younger siblings before the age of 6 years (<1 yr vs more), as those living more remote were more likely to have a lower education level and high sibling exposure.

RESULTS

Overall, 68% of participants were female, and the mean time since diagnosis of the cases was 9.4 years (Table 1). Table 2 shows the association between exposure to various animals and MS. In relation to farm-related variables, no associations were found for being a farmer (only two controls were farmers and no cases), living on a farm, exposure to specific farm animals (cows, sheep, horses, pigs, poultry, goats, sheep dogs, native animals or ferrets), or a variable that combined the individual farm animals into a combined exposure. Cases were more likely to be exposed to cats prior to symptom onset, and this strengthened after adjustment for smoking and sun exposure. However, no dose-response relationship was observed with an increasing duration of exposure to cats ($p=0.55$). We examined whether there was a specific age period where cat exposure was particularly important. The association became stronger when more years were added in (exposure prior to age 10 (OR 1.22 (0.79-1.90); prior to age 20 (OR 1.41 (0.87-2.29); prior to age 30 (1.69 (0.99-2.89))), but none were significant. Cat exposure 10 years prior to symptom onset was also not strongly associated (OR 1.50 (0.95-2.40), while cat exposure during adolescence (age12-18) was not associated at all (OR 1.12 (0.72-1.75). The magnitude of association of exposure to cats prior

onset did not differ by sex (p-value for interaction 0.77). Exposure to other pets (dogs, birds, Guinea pigs, rabbits) did not differ by case-control status and neither did other dog-related variables, including cumulative time with dogs (in years), the closeness of dogs to the participants (dog inside the home, outside or mixed) and whether the dog/s had suffered illness were not associated with MS. People reported a range of different illnesses for their dogs, including allergy/eczema, bowel problem, tumour, cancer/tumour, canine distemper, diabetes, heart disease, hydatids/worms, idiopathic hyperlipidaemia, kennel cough, kidney failure, mange, milk fever, parvo virus, rheumatoid arthritis, skin cancer, and tick paralysis. In relation to childhood exposures, we created variables including exposures prior to the age of 6, 10, 15 or 20 years and during puberty (age 12-18 years), but none were significantly associated with MS. We examined a range of remoteness of residence variables of which three are shown in Table 2. Although cases were slightly less likely to live more remotely, none of the remoteness variables was significantly associated with case status.

DISCUSSION

This population-based case-control study investigated whether farming and exposure to various pets or farm animals influenced the risk of MS. No associations were observed for farming, exposure to farm animals or remoteness of residence. We observed that people with MS were more likely to have had exposure to cats before diagnosis (OR 2.46 (1.17-5.18)), but there was no dose-response association with duration of exposure to cats, leaving it doubtful whether this was a true association. If there were a true association, our results seem to indicate that it is long-term exposure that mediates this effect.

We previously identified an association between occupational exposure to livestock and risk of CNS demyelination (OR 1.54 (1.03-2.33))[11], however we could not identify an association between exposure to livestock or specific animals in this study. Also, the significant association

between farming and risk of CNS demyelination in women was not identified since the prevalence of farming was very low. Even if this were a true association, then the impact in terms of population attributable fraction will be low.

In relation to exposure to cats, the literature has been inconsistent. It is unclear why this is the case, but possibly suggests that there is no true association. A recent case-control study in Norway suggests a protective effect of cat ownership (OR 0.56 (0.40-0.78)[12] supported by a 2001 case-control study by Ghadirian et al. identifying a similar relationship (OR 0.5 (0.3-0.8).[3] Several other studies found no association [5,11,13-15], and a study by Landtblom et al. showing an increased odds ratio for cat exposure and MS if the exposure was occupational in nature[13]. A positive correlation of dog exposure and MS risk has been reported as far back as 1978 in a small case control study in North America[16], and more recently in an Indian study[4]. Our results do not support this finding.

The case sample seemed similar to other populations with MS of north European ancestry for disease related features. Participation rates were high, reducing non-response bias, but it is possible that some selection bias may have occurred. Measurement error might have been an issue in relation to the exposure to animals, but the lifetime calendar was designed to improve recall, by using memorable life events such as schools and jobs as guideposts to improve recall. We tested a substantial number of pet and farming-related variables which increased the chance of identifying a false-positive association.

In conclusion, we have provided additional evidence that farming or exposure to farm animals do not alter the risk of MS.

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Table 1. Characteristics of MS cases and controls.

	MS cases (N=136)	Controls (N=272)
	n(%)	n(%)
Female sex, n (%)	92 (67.7)	184 (67.6)
Age, mean (SD) y	43.5 (9.3)	43.6 (9.2)
Age at diagnosis, mean (SD), y	34.6 (9.1)	
Duration of MS since diagnosis, mean (SD), y	9.4 (7.5)	
Duration since first symptoms, mean (SD), y	12.1 (8.0)	
EDSS score, mean (SD)	3.5 (2.2)	
Highest Education, n (%)		
University	25 (18)	41 (15)
Year 12/Tafe/trade	44 (33)	73 (27)
Year 10 or less	66 (49)	155 (58)
Remoteness classification at birth, n (%)		
Major City	3 (2.2)	8 (2.9)
Inner Regional	77 (56.6)	130 (48.0)
Outer Regional	50 (36.7)	126 (46.5)
Remote or very remote	6 (4.4)	7 (4.1)
Smoker before diagnosis, n (%)	85 (63)	143 (53)
History of infectious mononucleosis, n (%)	35 (25.7)	39 (14.3)
≥1 year of time with younger sibling before age 6 years	62 (46)	174 (64)
<i>HLA-DR15</i> genotype, n (%)	79 (58.1)	66 (28.5) *

* N=232

Table 2. Association between exposure to animals prior to disease onset, remoteness of residence and MS.

	Cases (N=136) n (%)	Controls (N=272) n (%)	Unadjusted Odds Ratio (95% CI)	p-value	Adjusted OR (95% CI)*	p-value
Farm animal exposure ever, prior to onset						
Lived at a farm	52 (38)	104 (38)	1.00 (0.63–1.58)	1.00	1.17 (0.72–1.90)	0.52
Cows	55 (40)	115 (42)	0.92 (0.59–1.43)	0.76	1.09 (0.68–1.73)	0.35
Sheep	46 (34)	86 (32)	1.13 (0.70–1.81)	0.63	1.28 (0.78–2.11)	0.33
Horses	40 (29)	73 (27)	1.13 (0.72–1.78)	0.59	1.27 (0.80–2.03)	0.31
Pigs	26 (19)	61 (22)	0.82 (0.49–1.37)	0.45	0.85 (0.51–1.44)	0.55
Poultry	67 (49)	140 (51)	0.91 (0.60–1.39)	0.67	1.02 (0.66–1.57)	0.94
Goats	13 (10)	23 (8)	1.14 (0.56–2.32)	0.71	1.29 (0.62–2.66)	0.50
Native animals	8 (6)	15 (6)	1.08 (0.43–2.71)	0.87	1.08 (0.42–2.75)	0.88
Ferrets	3 (2)	12 (4)	0.50 (1.41–1.77)	0.28	0.40 (0.11–1.44)	0.16
Farm animals combined**	73 (54)	148 (54)	0.97 (0.63–1.49)	0.88	1.12 (0.72–1.76)	0.60
Pet exposure ever, prior to onset						
Dogs	123 (90)	243 (89)	1.12 (0.57-2.19)	0.74	1.27 (0.63-2.56)	0.51
Cats	114 (84)	201 (74)	1.87 (1.09-3.24)	0.02	2.38 (1.31-4.30)	0.004
Birds	64 (47)	115 (42)	1.22 (0.80-1.86)	0.35	1.18 (0.76-1.83)	0.74
Guinea pigs	11 (8)	23 (8)	0.95 (0.43-2.10)	0.89	0.86 (0.39-1.96)	0.74
Rabbits	22 (16)	42 (15)	1.06 (0.61-1.84)	0.85	1.05(0.59-1.86)	0.17
Duration of cat exposure						
1 – 10 Years	37 (27)	53 (19)	2.22 (1.15 – 4.29)	0.02	3.05 (1.49 – 6.26)	0.002
11 – 20 Years	41 (30)	68 (25)	1.94 (1.03 – 3.67)	0.04	2.39 (1.21 – 4.75)	0.01
>20 Years	36 (26)	82 (30)	1.43 (0.76 – 2.70)	0.27	1.88 (0.95 – 3.73)	0.07
Test for trend				0.55		0.76
Average remoteness of residence at age 6-15 years						
Mean (SD)	2.44 (0.54)	2.51 (0.54)	0.77 (0.52–1.44)	0.20	0.78 (0.52–1.18)	0.24
Average remoteness of residence from birth prior to disease onset						
Mean (SD)	2.37 (0.46)	2.45 (0.48)	0.67 (0.42–1.07)	0.09	0.70 (0.43–1.13)	0.15
Average remoteness of residence in the 10 years prior to disease onset						
Mean (SD)	2.31 (0.52)	2.41 (0.56)	0.70 (0.47–1.04)	0.08	0.72 (0.47–1.11)	0.14

* Pet and farm animal exposure variables were adjusted for smoking prior to the onset (no, yes) and time spent in the sun

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5 during weekends and holidays before the age of 16 (≥ 2 -3 vs ≤ 1 -2 hrs/day).

6 * Remoteness variables were adjusted for education level and whether participants had exposure to younger siblings before
7 the age of 6 years (< 1 yr vs more), as those living more remote were more likely to have a lower education level and higher
8 sibling exposure.

9 ** Exposure to any of the above farm animals.

10 Remoteness Score: Average remoteness classification based residential location according to the Australian Bureau of
11 Statistics Classification: 1-Major City, 2-Inner Regional, 3-Outer Regional, 4-Remote, 5-Very Remote.

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Results: Exposure to other animals including livestock and farming, livestock, specific farm animals and remoteness of residence showed no significant association with MS risk. Exposure to cats prior to disease onset was associated with a greater risk of MS (Adjusted Odds Ratio 2.46 (1.17-5.18)) but without a clear dose-response (test for trend, $p=0.76$). ~~Exposure to other animals including livestock and farming and remoteness of residence showed no significant association with MS risk.~~

Conclusions: In contrast to other literature, farming as an occupation and exposure to livestock farm animals were not associated with MS. While we identified an association between cat exposure and MS, there was no dose-response relationship, and previous studies showed inconsistent results, leaving us to conclude that there is no strong evidence that exposure to cats is associated with MS.

~~In contrast to other literature, farming as an occupation and exposure to livestock was not associated with MS.~~

INTRODUCTION

Multiple Sclerosis (MS) is a complex disease of the central nervous system, and aetiological factors continue to be uncovered[1]. Contagious viral illnesses of pets, such as canine distemper, may potentially be associated with an increased risk of MS. However, immune modulation as a result of exposure to pets could also have a beneficial effect, in line with the “hygiene hypothesis”, which proposes that early life infections may down-regulate allergic and autoimmune disorders[2]. Inconsistent evidence exists regarding the association between exposure to pets and/or other animals and MS risk[3-5], but our group recently identified a positive association between farming and exposure to livestock and risk of central nervous system demyelination[6]. In a population based case-control study in Tasmania, we examined whether farming, and exposure to pets and farm animals prior to the onset of MS was associated with MS risk.

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explanation for the clinical features, and had to have relevant spinal cord abnormalities and changes on cerebral MRI consistent with demyelination. Controls were selected from the roll of registered electors, a comprehensive listing of the population maintained by the State Electoral Office of Tasmania. For each verified case, two control subjects were randomly selected and matched to the index case on sex and birth year. For the 136 cases included in the study, 272 eligible controls participated with a response rate of 76%.

All participants provided written consent with ethics approval being granted by the Human Research Ethics Committee of the Royal Hobart Hospital.

Measures

Data was collected by a partly self-completed life and lifetime calendar and a face-to-face interview was conducted by one of two research assistants between March 1999 and June 2001. For each year of their life participants indicated location of residence, the occupation they had, whether they lived at a farm, whether they had any farm animals and whether they had any pets at home that were owned by members of the household, including the type and number of pets. Regarding dog exposure, during the face-to-face interview, the interviewer would ask where the dog would mostly spend its time (mostly outdoors, more outdoors than indoors, more indoors than outdoors, mostly outdoors), how often the participant would cuddle, pat, nurse or stroke the dog (less than 3 times a day, 3-6 times a day, more than 6 times a day) and whether the dog had a disease at a certain stage of life. Residential locations were classified according to the Australian Standard Geographical Classification (ASGC) as a proxy for exposure to a rural environment.

Data Analysis

Using the yearly data from the calendar allowed the calculation of the time (in years) spent in contact with pets prior to the age of first symptom (and the same age for each matched control) or

average remoteness index and the calculation of exposure prior particular ages, to examine whether some age periods were of particular importance. Odds ratios and 95% confidence intervals were estimated by conditional logistic regression. We examined a number of factors as potential confounders as they were associated with MS in this dataset[7,8]. Farm and pet exposures were adjusted for smoking prior to the onset (no, yes) and time spent in the sun during weekends and holidays before the age of 16 ($\geq 2-3$ vs $\leq 1-2$ hrs/day) as e.g those with a cat were less likely to be smokers and more likely to be exposed to the sun. Remoteness variables were adjusted for education level and whether participants had exposure to younger siblings before the age of 6 years (<1 yr vs more), as those living more remote were more likely to have a lower education level and high sibling exposure.

RESULTS

Overall, 68% of participants were female, and the mean time since diagnosis of the cases was 9.4 years (Table 1). Table 2 shows the association between exposure to various animals and MS. In relation to farm-related variables, no associations were found for being a farmer (only two controls were farmers and no cases), living on a farm, exposure to specific farm animals (cows, sheep, horses, pigs, poultry, goats, sheep dogs, native animals or ferrets), or a variable that combined the individual farm animals into a combined exposure. Cases were more likely to be exposed to cats prior to symptom onset, and this strengthened after adjustment for smoking and sun exposure. However, no dose-response relationship was observed with an increasing duration of exposure to cats ($p=0.55$). We examined whether there was a specific age period where cat exposure was particularly important. The association became stronger when more years were added in (exposure prior to age 10 (OR 1.22 (0.79-1.90); prior to age 20 (OR 1.41 (0.87-2.29); prior to age 30 (1.69 (0.99-2.89)), but none were significant. Cat exposure 10 years prior to symptom onset was also not strongly associated (OR 1.50 (0.95-2.40), while cat exposure during adolescence (age12-18) was not associated at all (OR 1.12 (0.72-1.75). The magnitude of association of exposure to cats prior

onset did not differ by sex (p-value for interaction 0.77). Exposure to other pets (dogs, birds, Guinea pigs, rabbits) did not differ by case-control status and neither did other dog-related variables, including cumulative time with dogs (in years), the closeness of dogs to the participants (dog inside the home, outside or mixed) and whether the dog/s had suffered illness were not associated with MS. People reported a range of different illnesses for their dogs, including allergy/eczema, bowel problem, tumour, cancer/tumour, canine distemper, diabetes, heart disease, hydatids/worms, idiopathic hyperlipidaemia, kennel cough, kidney failure, mange, milk fever, parvo virus, rheumatoid arthritis, skin cancer, and tick paralysis. ~~In relation to farm-related variables, no associations were found for being a farmer (only two controls were farmers and no cases), living on a farm, exposure to specific farm animals (cows, sheep, horses, pigs, poultry, goats, sheep dogs, native animals or ferrets), or a variable that combined the individual farm animals into a combined exposure. In relation to childhood exposures, we created variables including exposures prior to the age of 6, 10, 15 or 20 years and during puberty (age 12-18 years), but none were significantly associated with MS.~~ In relation to childhood exposures, we created variables including exposures prior to the age of 6, 10, 15 or 20 years and during puberty (age 12-18 years), but none were significantly associated with MS. We examined a range of remoteness of residence variables of which three are shown in Table 2. Although cases were slightly less likely to live more remotely, none of the remoteness variables was significantly associated with case status.

DISCUSSION

This population-based case-control study investigated whether farming and exposure to various pets or farm animals influenced the risk of MS. No associations were observed for other pets, farming, exposure to farm animals or remoteness of residence. We observed that people with MS were more likely to have had exposure to cats before diagnosis (OR 2.46 (1.17-5.18)), but there was no dose-response association with duration of exposure to cats, leaving it doubtful whether this was a true association. If there were a true association, our results seem to indicate that it is long-term

exposure that mediates this effect. ~~No associations were observed for other pets, farm animals or remoteness of residence.~~

We previously identified an association between occupational exposure to livestock and risk of CNS demyelination (OR 1.54 (1.03-2.33))[11], however we could not identify an association between exposure to livestock or specific animals in this study. Also, the significant association between farming and risk of CNS demyelination in women was not identified since the prevalence of farming was very low. Even if this were a true association, then the impact in terms of population attributable fraction will be low.

In relation to exposure to cats, the literature has been inconsistent. It is unclear why this is the case, but possibly suggests that there is no true association. A recent case-control study in Norway suggests a protective effect of cat ownership (OR 0.56 (0.40-0.78))[12] supported by a 2001 case-control study by Ghadirian et al. identifying a similar relationship (OR 0.5 (0.3-0.8)).[3] Several other studies found no association [5,11,13-15], and a study by Landtblom et al. showing an increased odds ratio for cat exposure and MS if the exposure was occupational in nature[13]. A positive correlation of dog exposure and MS risk has been reported as far back as 1978 in a small case control study in North America[16], and more recently in an Indian study[4]. Our results do not support this finding.

The case sample seemed similar to other populations with MS of north European ancestry for disease related features. Participation rates were high, reducing non-response bias, but it is possible that some selection bias may have occurred. Measurement error might have been an issue in relation to the exposure to animals, but the lifetime calendar was designed to improve recall, by using memorable life events such as schools and jobs as guideposts to improve recall. We tested a

substantial number of pet and farming-related variables which increased the chance of identifying a false-positive association.

In conclusion, we have provided additional evidence that farming or exposure to farm animals do not alter the risk of MS. while we identified an association between cat exposure and MS, there was no dose-response relationship, and previous studies showed inconsistent results, leaving us to conclude that there is no strong evidence that exposure to cats is associated with MS. Furthermore, we have provided additional evidence that exposure to other animals including farm animals does not alter risk of MS.

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Table 1. Characteristics of MS cases and controls.

	MS cases (N=136)	Controls (N=272)
	n(%)	n(%)
Female sex, n (%)	92 (67.7)	184 (67.6)
Age, mean (SD) y	43.5 (9.3)	43.6 (9.2)
Age at diagnosis, mean (SD), y	34.6 (9.1)	
Duration of MS since diagnosis, mean (SD), y	9.4 (7.5)	
Duration since first symptoms, mean (SD), y	12.1 (8.0)	
EDSS score, mean (SD)	3.5 (2.2)	
Highest Education, n (%)		
University	25 (18)	41 (15)
Year 12/Tafe/trade	44 (33)	73 (27)
Year 10 or less	66 (49)	155 (58)
Remoteness classification at birth, n (%)		
Major City	3 (2.2)	8 (2.9)
Inner Regional	77 (56.6)	130 (48.0)
Outer Regional	50 (36.7)	126 (46.5)
Remote or very remote	6 (4.4)	7 (4.1)
Smoker before diagnosis, n (%)	85 (63)	143 (53)
History of infectious mononucleosis, n (%)	35 (25.7)	39 (14.3)
≥1 year of time with younger sibling before age 6 years	62 (46)	174 (64)
<i>HLA-DR15</i> genotype, n (%)	79 (58.1)	66 (28.5) *

* N=232

Table 2. Association between exposure to animals prior to disease onset, remoteness of residence and MS.

	Cases (N=136) n (%)	Controls (N=272) n (%)	Unadjusted Odds Ratio (95% CI)	p-value	Adjusted OR (95% CI)*	p-value
Farm animal exposure ever, prior to onset						
Lived at a farm	52 (38)	104 (38)	1.00 (0.63–1.58)	1.00	1.17 (0.72–1.90)	0.52
Cows	55 (40)	115 (42)	0.92 (0.59–1.43)	0.76	1.09 (0.68–1.73)	0.35
Sheep	46 (34)	86 (32)	1.13 (0.70–1.81)	0.63	1.28 (0.78–2.11)	0.33
Horses	40 (29)	73 (27)	1.13 (0.72–1.78)	0.59	1.27 (0.80–2.03)	0.31
Pigs	26 (19)	61 (22)	0.82 (0.49–1.37)	0.45	0.85 (0.51–1.44)	0.55
Poultry	67 (49)	140 (51)	0.91 (0.60–1.39)	0.67	1.02 (0.66–1.57)	0.94
Goats	13 (10)	23 (8)	1.14 (0.56–2.32)	0.71	1.29 (0.62–2.66)	0.50
Native animals	8 (6)	15 (6)	1.08 (0.43–2.71)	0.87	1.08 (0.42–2.75)	0.88
Ferrets	3 (2)	12 (4)	0.50 (1.41–1.77)	0.28	0.40 (0.11–1.44)	0.16
Farm animals combined**	73 (54)	148 (54)	0.97 (0.63–1.49)	0.88	1.12 (0.72–1.76)	0.60
Pet exposure ever, prior to onset						
Dogs	123 (90)	243 (89)	1.12 (0.57–2.19)	0.74	1.27 (0.63–2.56)	0.51
Cats	114 (84)	201 (74)	1.87 (1.09–3.24)	0.02	2.38 (1.31–4.30)	0.004
Birds	64 (47)	115 (42)	1.22 (0.80–1.86)	0.35	1.18 (0.76–1.83)	0.74
Guinea pigs	11 (8)	23 (8)	0.95 (0.43–2.10)	0.89	0.86 (0.39–1.96)	0.74
Rabbits	22 (16)	42 (15)	1.06 (0.61–1.84)	0.85	1.05(0.59–1.86)	0.17
Duration of cat exposure						
1 – 10 Years	37 (27)	53 (19)	2.22 (1.15 – 4.29)	0.02	3.05 (1.49 – 6.26)	0.002
11 – 20 Years	41 (30)	68 (25)	1.94 (1.03 – 3.67)	0.04	2.39 (1.21 – 4.75)	0.01
>20 Years	36 (26)	82 (30)	1.43 (0.76 – 2.70)	0.27	1.88 (0.95 – 3.73)	0.07
Test for trend				0.55		0.76
Average remoteness of residence at age 6-15 years						
Mean (SD)	2.44 (0.54)	2.51 (0.54)	0.77 (0.52–1.44)	0.20	0.78 (0.52–1.18)	0.24
Average remoteness of residence from birth prior to disease onset						
Mean (SD)	2.37 (0.46)	2.45 (0.48)	0.67 (0.42–1.07)	0.09	0.70 (0.43–1.13)	0.15
Average remoteness of residence in the 10 years prior to disease onset						
Mean (SD)	2.31 (0.52)	2.41 (0.56)	0.70 (0.47–1.04)	0.08	0.72 (0.47–1.11)	0.14

* Pet and farm animal exposure variables were adjusted for smoking prior to the onset (no, yes) and time spent in the sun

Comment [IVdM1]: Non-tracked change: This section was below pet exposure and has now been shifted to the top

during weekends and holidays before the age of 16 (≥ 2 -3 vs ≤ 1 -2 hrs/day).

* Remoteness variables were adjusted for education level and whether participants had exposure to younger siblings before the age of 6 years (< 1 yr vs more), as those living more remote were more likely to have a lower education level and higher sibling exposure.

** Exposure to any of the above farm animals.

Remoteness Score: Average remoteness classification based residential location according to the Australian Bureau of Statistics Classification: 1-Major City, 2-Inner Regional, 3-Outer Regional, 4-Remote, 5-Very Remote.

CI, Confidence Interval

Conflict of interest: There are no relevant conflicts of interest to declare.

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