Sero–Epidemiology of Brucellosis in Organized Cattle and Buffaloes in Punjab (India)

Article *in* Advances in Animal and Veterinary Sciences · November 2013

CITATIONS
12

READS
502

8 authors, including:

Malik Raies
Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir
16 PUBLICATIONS **55** CITATIONS
SEE PROFILE

Gursimran Filia
Guru Angad Dev Veterinary and Animal Sciences University
52 PUBLICATIONS **170** CITATIONS
SEE PROFILE

Pritam Sidhu
Kansas State University
62 PUBLICATIONS **487** CITATIONS
SEE PROFILE

Tawheed Ahmad Shafi
Maharashtra Animal & Fishery Sciences University
21 PUBLICATIONS **35** CITATIONS
SEE PROFILE

Advances in Animal and Veterinary Sciences. 1 (3S): 5 – 8
Special Issue – 3 (Epidemiology and Animal Disease Investigations)
http://www.nexusacademicpublishers.com/journal/4

Research Article

Sero–Epidemiology of Brucellosis in Organized Cattle and Buffaloes in Punjab (India)

Malik Raies Ul Islam*[1], Mohinder Pratap Gupta[2], Gursimran Filia[2], Pritam Kaur Sidhu[2], Tawheed Ahmed Shafi[3], Suhail Ahmed Bhat[4], Sayed Ashaq Hussain[3], Radya Mustafa[5]

[1]KVK Malangpora, S. K. University of Agricultural Sciences and Technology, Kashmir– 192301; [2]Animal Disease Research Centre; [3]Veterinary Medicine; [4]Veterinary Parasitology, Guru Angad Dev Veterinary and Animal Sciences University Ludhiana –141004, Punjab–India; [5]Epidemiology and Public Health, Omar al Mukhtar University Albayda Libya.
*Corresponding author: malikrayees@gmail.com; malik_rayees@yahoo.com

ARTICLE HISTORY

Received: 2013–10–08
Revised: 2013–10–15
Accepted: 2013–10–20

Key Words: Animal, Brucellosis, Buffaloes, Cattle, Epidemiology, ELISA, Sero–prevalence

ABSTRACT

The present cross sectional study was carried out to assess the current status and epidemiology of brucellosis in organized cattle and buffaloes. A stratified random sampling approach was used to select the sample size. In the first strata, villages were selected followed by selection of dairy farms in the second strata. A total of 39 villages were selected using survey tool box and from these selected villages 1203 animals (575 cattle and 628 buffaloes) were screened for anti–brucella antibodies using indirect Enzyme Linked Immuno–sorbent Assay (I–ELISA) from December 2010 to March 2012. In the study population, an overall sero–prevalence of brucellosis was observed as 21.36%. Statistically significant ($p<0.01$) differences in prevalence of brucellosis among cattle (28.17%) and buffaloes (15.12%) and with respect to sex (Male, 1.81% and Female 28.69%) were observed. The sero–prevalence of brucellosis was significantly ($p<0.01$) higher in animals with history of abortion than that of (21.70%) without such histories (23.01%).

ARTICLE CITATION: Ul Islam, MR, Gupta MP, Filia G, Sidhu PK, Shafi TA, Bhat SA, Hussain SA and Mustafa R (2013). Sero–epidemiology of brucellosis in organized cattle and buffaloes in Punjab (India). *Adv. Anim. Vet. Sci.* 1 (3S): 5 – 8.

INTRODUCTION

Brucellosis is an important bacterial zoonosis caused by different species of genus *Brucella*. The genus *Brucella* has been classified into several species on the basis of preference of each species to its natural host that also serves its reservoir (Quinn et al., 1994). Bovine brucellosis is mainly caused by *Brucella abortus* (*B. abortus*) and less frequently by *B. melitensis* (OIE, 2008). Brucellosis is transmitted by direct or indirect contact with infected materials like aborted fetuses, after births, infected animals, and oral route serves as the main portal of entry of this agent (Quinn et al., 1994). Brucellosis is endemic in most parts of the world especially the developing world including India and poses a serious public health threat (Kumar et al., 2009; Dhama et al., 2013) and leads to significant economic losses (Tiwari et al., 2013). The disease in animals is characterized by abortion in last trimester of pregnancy, retention of after births, decreased milk production and infertility in females and orchitis in males (Radostitis et al., 2000; OIE, 2008). Semen used for artificial insemination from infected bulls has a great potential of spreading the disease (Eshetu et al., 2005).

In India, the disease is showing an upward trend and there has been a staggering increase in the prevalence and incidence of the disease. The changing husbandry practices from traditional to modern livestock rearing, coupled with stocking of more number of animals per unit area have resulted in spatial clustering of both the infection and the disease. The situation is further complicated by unrestricted movement of animals, desperate sale of positive animals, ban on cow slaughter due to religious reasons and lack of strict brucellosis control strategy. Serological evidence suggests that brucellosis is highly endemic in most parts of India (Mehra et al., 2000; Sandhu et al., 2001; Shringi et al., 2002; Sarumathi et al., 2003; Mahato et al., 2004; Singh et al., 2004; Mittal et al., 2005; Kumar et al., 2009). The sero–prevalence of brucellosis in cattle ranged from 0.3% in Himachal Pradesh (Renukaradhya et al., 2002) to 56.2% in Assam (Chakraborty et al., 2000). In the states of Uttar Pradesh and Delhi Sharma et al. (1979) carried out sero–epidemiological investigation on brucellosis and reported a sero–positivity of 6.37 % in cattle and 4.9 % in buffaloes. The variation could be due to use of non random sampling techniques (Dhand et al., 2005). Most of these studies have used purposive or convenience sampling methods (Dhand et al., 2005) and all the information generated from these surveys cannot be generalized to the target population. Moreover, these studies have used conventional tests for detection of anti–brucella antibodies in serum. The present study was, therefore, carried out to study the current status and epidemiology of brucellosis in organized cattle and buffaloes using indirect enzyme linked immunosorbent assay.

MATERIALS AND METHODS

Study Area

Punjab is the north–western state of India bordering with Pakistan on the west and situated between the 29.30° N to 32.32° N latitude and 73.55° E to 76.50°E longitude. Approximately 70% of human population lives in villages and agriculture is the main occupation. Murrah and Niliravi breeds of buffaloes and cross bred Holstein Frisian cattle are mostly

ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)

reared in this region. The Murrah buffalo breed is also called the black gold of this region.

Study Animals

The study was conducted on organized cattle and buffaloes. Holstein Frisian cattle and Murrah buffaloes were the breeds studied. Murrah breed of buffalo is jet black in colour and has short curved horns.

Design of the Study

The cross–sectional study was carried out from December, 2010 to March, 2012. Indirect Enzyme Linked Immunosorbent Assay (I–ELISA) and questionnaire were used to evaluate the sero–prevalence of brucellosis and host factors associated with the occurrence of brucellosis respectively. Host factors studied were species, breed, age, sex and history of abortion.

Determination of Sample Size

A stratified random sampling approach was used to select the sample size. In the first strata villages were selected followed by selection of dairy farms. A total of 39 villages were selected using survey tool box and from each village an organized dairy farm was selected using table of random numbers. A sample size of 1203 animals was selected using Win episcope –2 (95% confidence level, and ± 5% desired level of accuracy).

Sample Collection

Blood samples were aseptically collected from the selected 1203 (575 cattle and 628 buffaloes) animals by jugular vein–puncture. About 5–10 ml of blood was collected in plain tubes without any anticoagulant. The blood samples were kept on ice immediately and until transported to laboratory. Serum was separated from clotted blood by centrifugation at 3000 rpm for 5 minutes and stored at – 20ºC till further use.

Serological Testing

Indirect –Enzyme Linked Immuno–Sorbent Assay (I–ELISA)

The test was performed as per the manufacturer's protocol (Bionote, Korea). All the reagents of the kit and serum samples were brought to room temperature. Working dilutions of the reagents were prepared as per instructions of the manufacturer. Test samples were diluted 1:50 with sample diluent and 100µl of each diluted sample was transferred to a well in the micro–titre plate. Controls were run using 100µl of undiluted strong positive, weak positive and negative control into the designated wells. Mixing of plate contents was ensured by incubating the plate for 5 minutes in an orbital shaker followed by incubation of plate at 37 °C for one hour. After incubation the contents were discarded and the plate was washed five times with the wash buffer (PBS–Tween20). The plate was blot dried and 100µl of diluted enzyme conjugate was transferred to each well and incubated for 30 minutes. The contents of the plate were discarded followed by washing with wash buffer five times. After blot drying the plate, 100µl of ready to use substrate was transferred to each well followed by incubation for 15 minutes at room temperature. The reaction was stopped by addition of 100µl of stopping solution (1N sulphuric acid) to each well and absorbance of the wells was read at 450nm along with the reference wavelength of 620nm. The optical density (OD) values were used to calculate the percent positivity as shown in the equation. The test sera were categorised as positive or negative based upon the percent positivity value. Samples having percent positivity value 25 or above (%P ≥ 25) were categorised as positive and below 25 as.

Statistical Analysis

Data from laboratory test and signalment of each animal were stored in excel spread sheet. The data were analyzed using Chi Square (χ^2) test and Fisher's exact test, wherever needed.

RESULTS

Sero–prevalence of brucellosis in the Punjab state was estimated on the basis of results obtained by I–ELISA. An overall sero–prevalence of 21.36% (257/1203) of brucellosis was observed in the study population. Species, age, sex and history of abortion were the host factors studied. The prevalence of brucellosis among cattle and buffaloes was 28.17% (162/575) and 15.12% (95/628), respectively and the differences observed were statistically significant ($p<0.01$). Statistically significant ($p<0.01$) differences with respect to susceptibility to infection were also observed among male (1.81%, 1/55) and female (28.69%, 256/1148) animals in the present study (table 1).

Table 1: Species, Sex and age wise Prevalence of Brucellosis

S. No		Tested	Positive	% Positive
Species	Cattle	575	162	28.17
	Buffaloes	628	95	15.12
Sex	Male	55	1	1.81
	Female	1148	256	22.29
Age	‹ 3	451	39	8.64
	3 – 7	525	141	26.85
	› 7	227	77	39.92

Over all ser–prevalence, 257/1203 = 21.36%

Another host factor studied was the age of the animals. The prevalence of brucellosis was higher in animals of age ›7years (39.92%, 77/227), followed by animals in the age groups of 3–7 years (26.85%, 141/525) and least in animals of ‹3 years (8.64%, 39/451) of age (table 1). The differences in the prevalence of the disease among these three age groups were statistically significant ($p<0.01$), with animals in the age group of ›7 years being the most susceptible.

With regard to history of abortion male animals and animals up to two years of age were excluded from the data so as to avoid bias. Of the remaining animals, 11.13% (91/817) were having history of abortion. Of these aborted animals, 75.82% (69/91) were sero–positive. The sero–prevalence of brucellosis was significantly ($p<0.01$) higher in animals with a history of abortion than in those (21.70%, 158/728) without such histories (23.01%, 188/817). Out of the total 91 cases of abortion recorded in cattle and buffaloes, 65 cases of abortions occurred in the third trimester, 24 in the second and 2 in the first trimester of gestation. The sero–prevalence of brucellosis was 1.09%, 17.58% and 57.14% in animals with history of abortion in first, second and third trimesters of pregnancy respectively, but the differences were statistically non significant ($p<0.05$). Out of 91 aborted animals, 72.52% (66) had history of retention of placenta and of these 55 were cows and 11 were buffaloes (table 2).

Table 2: Brucellosis in aborted animals and retained cases of placenta

Species	Cattle		Buffaloes	
	Total	Positive	Total	Positive
Aborted	70	52	21	17
Retention of Placenta	55	39	11	7

Trimester of pregnancy at the Time of Abortion

S. No	Aborted	Positive
1st	2	1
2nd	24	16
3rd	65	52

ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)

DISCUSSION

Brucellosis remains one of the major public health concerns throughout the developing world (Karthik et al., 2013). In India, the disease prevalence has shown an unprecedented increase ever since it was first recognized in 1942. The disease has been reported from most of the domestic animals and humans (Renukaradya et al., 2002). The prevalence of disease varies greatly from country to country and across different regions with in a country (Acha and Szyfers, 2001).

Sero–prevalence of bovine brucellosis has been assessed at different times from different regions of the country (Mehra et al., 2000; Shringi et al., 2002; Sarumathi et al., 2003; Mahato et al., 2004; Mittal et al., 2005; Kumar et al., 2009). An overall sero–prevalence of 21.36% was observed in the present study, which is higher than that reported by Dhnad et al. (2005). The present study was carried out in organized dairy farms (cattle and buffaloes), which tend to have more animals per unit area. Large herd size enhances the exposure potential through increased contact between infected and non infected animals, thereby prompting transmission of the organism (Hellman et al., 1984). More ever stocking densities have been found to be important determinant for *Brucella* transmission (Omer et al., 2002). The disease is chronic in nature and infection may go unnoticed especially in case of heifers, males and young animals, which can lead to spatial clustering of cases.

A statistically significant difference in sero–prevalence of brucellosis in cattle and buffaloes was observed. This finding was in concurrence with that of Chatterjee et al. (1986), but differed from that of Saini et al. (1992) who reported higher disease prevalence in buffaloes. Dhand et al. (2005) reported that cattle and buffaloes are equally susceptible to the infection. Cross bred cattle are more susceptible to stress conditions than buffaloes; the genetic differences especially in innate immune system may also be the possible reason for variation in sero–prevalence between the two species. Moreover, the stocking densities are higher in cattle farms compared buffalo farms, which increase the chances of exposure due to increased contact between infected and uninfected animals.

Generally male and female animals are equally susceptible to brucellosis, however statistically significant differences in susceptibility to brucellosis between male and female animals were observed. A higher sero–prevalence of brucellosis in female animals has been reported by various studies (Asfaw et al., 1998; Muma et al., 2007; Tolosa et al., 2008; Bayemi et al., 2009). The differences observed may be due to the fact that only 55 males were available for sampling in the study area, as most of the farmers opt for artificial breeding method. Another aspect is that female animals are kept for longer in a particular herd and are stocked together compared to male animals which are individually housed, thereby increasing chances of exposure in females (Mekonnen et al., 2010).

With respect to age, the sero–prevalence of brucellosis was higher in animals of age group >7 years followed by animals in the age group of 3–7 years and least in animals of < 3 years of age and the differences observed were statistically significant. The results of the present study suggest that animals of more than 3 years of age are more likely to become sero–positive to brucellosis. Similar observations have been made by other workers (Botha et al., 1989; Silva et al., 2000; Sarumathi et al., 2003; Amin et al., 2005). Lower prevalence of brucellosis in young ones could be due to resistance of young animals to infection (Paul, 1980). Dhand et al. (2005) suggested that with passage of time animals are more likely to be exposed to the bacteria and contract the disease. However, Kazi et al. (2005) reported that high prevalence of brucellosis among old animals might be related to maturity with advancing age, thereby the organism may have propagated to remain as latent infection or it may cause disease. Although susceptibility to brucellosis increases with age, it seems to be commonly associated with sexual maturity than age (Radiostitis et al., 2000).

Outbreaks of bovine brucellosis are associated with abortion in last trimester of gestation, retention of after births, production of weak new born calves and infertility in animals (OIE, 2008). With respect to abortion history, female animals greater than two years of age were included in the analysis to avoid bias. 11.13% of animals (91/817) had a history of abortion and of these 75.82% (69/91) were sero–positive. Statistically significant differences in sero–prevalence between animals with history of abortion and those without such histories were observed, these findings were in concurrence with that of Sandhu et al. (2001), who reported a higher sero–prevalence of brucellosis in animals with history of abortion. Though other infectious and non–infectious causes prevalent in the study area could also have contributed to abortion, however the results of the present study indicated that brucellosis was a major cause of abortion in dairy animals. Most of these abortions took place in the last trimester of gestation, followed by second trimester and least in first trimester. The higher incidence of abortion in third trimester may be due to the fact that uterine environment becomes conducive for growth of *Brucellae* due to production of erythritol, which in turn causes damage to placenta and abortion(Radostits et al., 2000). Retention of placenta is a common sequel to abortion in brucellosis (Radostits et al., 2000). The prevalence of abortion and retention of placenta recorded in the present study was higher than that reported by Tesfaye et al. (2011). Most of these abortions and retention of after births were recorded in cattle, which may be due to fact that cow slaughter is banned in India and farmers usually keep the infected animals in the main herd or may sell these infected animals to another farmer without disclosing any previous history of abortion to the buyer, thus becoming a source of introduction of infection in a new premises, which sometimes leads to abortion storms.

CONCLUSION

A higher prevalence of brucellosis was recorded in the present study, which is a serious public and animal health threat. Therefore, a constant monitoring system needs to be in place to study the changes in the disease dynamics so that the control strategies can be manipulated to bring down the incidence and prevalence of the disease to a justifiable level. Prevalence was statistically different in cattle and buffaloes, but both species need to be taken into consideration while implementing the control programmes.

ACKNOWLEDGEMENT

The support provided by Animal Disease Research Centre and the University authorities is highly appreciated.

CONFLICT OF INTEREST

The authors have no conflict of interest

REFERENCES

Acha PU and Szyfers B (2001). Zoonosis and Communicable Disease Common to Man and Animals (3rd ed.) Pan America Health Organization, Washington, DC. 40–296.

Amin KMR, Rahman MB, Rahman MS, Han,JC, Park JH and Chae JS (2005). Prevalence of *Brucella* antibodies in sera of cows in Bangladesh. J. Vet. Sci. 6: 223–226.

Asfaw Y, Molla B, Zessin K and Tegegn A (1998). A cross sectional study on bovine brucellosis and test performance in intra and periurban dairy production system in and around Addis Ababa. B. Anim. Health Prod. Afr. 46: 217–224.

ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)

Bayemi PH, Webb EC, Nsongka MV, Unger H and Njakoi H (2009). Prevalence of Brucella abortus antibodies in serum of Holstein cattle in Cameroon. Trop. Anim. Health. Pro.. 41: 141–144.

Botha CJ and Williamson CC (1989). A serological survey of bovine brucellosis in four districts of Bophuthatswana. J. S. Afr. Vet. Assoc. 60: 50.

Chakraborty M, Patgiri GP and Sarma DK (2000). Use of Rose Bengal Plate Test, Serum Agglutination Test and Indirect–ELISA for detecting brucellosis in bovines. Indian J. Comp. Microbiol. Immunol. Infect. Dis. 21: 24–25.

Chatterjee AD, Mondal P, Bidyanta JC, Chakraborty MK, Nandy AK and Sen GP (1986). Studies on seroprevalence on bovine brucellosis in rural West Bengal. Indian Vet. J. 63: 95–100.

Dhama K, Verma AK, Tiwari R, Chakraborty S, Vora K, Kapoor S, Deb R, Karthik K, Singh R, Munir M, Natesan S (2013). A perspective on applications of Geographical Information System (GIS); an advanced tracking tool for disease surveillance and monitoring in Veterinary epidemiology. Advances in Animal and Veterinary Sciences. 1(1): 14–24.

Dhand NK, Gumber S, Singh BB, Aradhana, Bal MS, Kumar H, Sharma DR, Singh J and Sandhu KS (2005). A study on the epidemiology of brucellosis in Punjab (India) using Survey Toolbox. Rev. Sci Tech. OIE. 24 (3): 879–85.

Eshetu Y, Kassahun J, Abebe P, Beyene M, Zewdie B and Bekele A (2005). Seroprevalence Study of Brucellosis on Dairy Cattle in Addis Ababa, Ethiopia. B. Anim. Health Prod. Afr. 53: 211–214.

Hellman E, Staak C and Baumann M (1984). Bovine brucellosis among two different cattle populations in Bahr el Ghazal Province of Southern Sudan. Tropenmed Parasitology. 35: 123–26.

Kazi M, Amin R, Rahman MB, Rahman MS, Han J, Park J and Chae J (2005). Prevalence of *Brucella* antibodies in sera of cows in Bangladesh. J. Vet. Sci. 6: 223–26.

Karthik K, Rathore R, Verma AK, Tiwari R, Mahima and Dhama K (2013). Brucellosis– still it stings? Livestock Technology 2(10): 8–10

Kumar N, Pal BC, Yadav SK, Verma AK, Jain U and Yadav G (2009). Prevalence of Bovine Brucellosis in Uttar Pradesh, India. Journal of Veterinary Public Health, 7(2): 129–131.

Mahato G, Sharma K and Mahantam PN (2004). Comparative evaluation of serological tests for detection of Brucellosis in bovine. Indian J. Vet. Med. 24: 46.

Mehra KN, Dhanesar NS and Chaturvedi VK (2000). Sero–prevalence of brucellosis in bovines of Madhya Pradesh. Indian Vet. J. **77:** 571–73.

Mekonnen H, Kalayou S and Kyule M (2010). Serological survey of bovine brucellosis in barka and arado breeds (Bos indicus) of Western Tigray, Ethiopia. Prev. Vet. Med. 94: 28–35.

Mittal V, Kumar M and Ambwani T (2005). Seroepidemiological pattern of brucellosis among livestock of district Udham Singh Nagar in Uttaranchal. Indian J. Vet. Med. 25: 28–32.

Muma JB, Toft N, Oloya J, Lund A, Nielsen K, Samui K and Skjerve E (2007). Evaluation of three serological tests for brucellosis in naturally infected cattle using latent class analysis. Vet. Microbiol. 125: 187–92.

OIE (2008). Manual of the Diagnostic Tests and Vaccines for Terrestrial animals, vol. 1. 5th edition Office International Des Epizooties, Paris, France. 409–438.

Omer MK, Skjerve E, Holstad G and Woldehiwete Z (2002). Prevalence of antibodies to *Brucella* spp. in cattle, sheep, goats, horses and camels in the State of Eritrea; influence of husbandry systems. J. Infection. 125: 447–53.

Paul A (1980). The Epidemiology of bovine brucellosis. Adv. Vet. Sci. Comp. Med. 24: 75.

Quinn PJ, Carter M, Markey B and Carter GR (1994). Clinical Veterinary Microbiology. Wolfe Publishing, London UK. 261–267

Radostits OM, Gay CC, Blood DC and Hinchcliff KW (2000). Diseases caused by *Brucella* spp. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. Ninth edition, London, Harcourt Publishers Limited.

Renukaradhya GJ, Isloor S and Rajasekhar M (2002). Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis. IndianVet. Microbiol. 90: 183–95.

Saini SS, Sharma JK, Kwatra MS (1992). Assessment of some management factors responsible for prevalence of brucellosis among traditionally managed animal population of Punjab. Indian J. Anim. Sci. 62 (9): 832–834

Sandhu KS, Filia G, Sharma DR, Dhand NK, Singh J and Saini SS (2001). Prevalence of brucellosis among dairy animals of Punjab. Indian J. Comp. Microbiol. Immunol. Infect. Dis. 22: 160–161.

Sarumathi C, Reddy TV and Sreedevi B (2003). Serological survey of bovine brucellosis in Andhra Pradesh. Indian J. Dairy Sci. 56: 408–10.

Sharma VD, Sethi MS, Yadav MP and Dube DC (1979). Sero–epidemiologic investigations on brucellosis in the states of Uttar Pradesh and Delhi (India). Int. J. Zoonoses. 6: 75–81.

Shringi BN, Sharma S and Sharma.KN (2002). Comparative study of conventional serological test for the diagnosis of brucellosis. Indian J. Anim. Sci. 72: 553–54.

Silva I, Dangolla A and Kulachelvy K (2000). Seroepidemiology of *Brucella* abortus infection in bovids in Sri Lanka. Prev. Vet. Med. 46: 51–59.

Singh G, Sharma DR. and Dhand NK (2004). Seroprevalence of bovine brucellosis in Punjab. Indian Vet. J. 81: 620–23.

Tesfaye G, Tsegaye W, Chanie M and Abinet F (2011). Seroprevalence and associated risk factors of bovine brucellosis in Addis Ababa dairy farms. Trop Anim. Health Pro. 43: 1001–1005.

Tiwari R, Sharma MC, Mishra KK and Singh BP (2013). Economic impacts of infectious diseases of livestock. Ind. J. Ani. Sci. 83: 316–320.

Tolosa T, Regassa F and K Belihu (2008). Seroprevalence Study Of Bovine Brucellosis In extensive management System In Selected Sites Of Jimma Zone, Western Ethiopia. B. Anim. Health Prod. Afr. 56(1): 25–37.

ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)